INFECTION AND IMMUNITY, May 1974, p. 971-973

Vol. 9, No. 5
Printed in U.S.A.

Lectins Detecting Group C Streptococci

FRITZ OTTENSOOSER, YASUZO NAKAMIZO, MAROTO SATO, YASUSHI MIYAMOTO, AND KINJIRO TAKIZAWA

Faculty of Medicine, University of São Paulo, São Paulo, Brazil, and Japanese Research Committee of Scarlet Fever, Department of Infectious Disease, Tokyo Metropolitan EBARA Hospital, Tokyo, and Department of Bacteriology, Kanagawa Prefectural Institute of Hygienic Sciences, Japan

Received for publication 12 October 1973

Lectin of *Wisteria floribunda* specifically agglutinates group C streptococci. The reaction is strongly inhibited by *N*-acetyl-D-galactosamine, slightly by lactose, and not at all by galactose. The utility of the simple test of the lectin with group C streptococci is discussed.

Lectins (plant agglutinins) have been extensively studied as to their reactions with blood cells. Some of them (2, 3, 10, 11) are now in practical use, being easier to obtain, cheaper, and more specific than antisera produced by immunization of rabbits.

Lectins may combine specifically with defined sugar structures. This has often been applied to characterize the specificity of cell surfaces which is based on sugars. Landsteiner (8) was the first to show that simple substances similar to, or identical with, the immunologically determinant group are able to combine with the corresponding antibody and thereby to inhibit competitively the reaction between cellular antigen and antibody.

The firmness with which simple substances are bound, that is, the closeness of relationship of the simple substance to the determinant antigen structure, is inversely proportional to the concentration required to produce inhibition. In the field of blood groups, this was seen by Watkins and Morgan (13) when they found that L-fucose specifically inhibited the agglutination of O cells by eel anti-O (H) serum. Similarly, inhibition of the agglutination of A cells by plant anti-A obtained from *Vicia cracca* and lima beans gave the first clue to the part played by *N*-acetyl-D-galactosamine (9) in A specificity.

Some years ago, lectins and reagents of animal origin were detected which, following the rules of chemospecificity, are very useful for bacterial classification. Köhler and Prokop (5, 7) described an efficient method, complementary to the usual ones for identifying group C hemolytic streptococci. They found that the agglutinin ("protectin") from the albumin gland of *Helix pomatia* is highly specific. These authors differentiated the C streptococci also with the lectin of *Dolichos hiflorus* (6) and demonstrated that the specific reactions of the protectin and the lectin are determined by *N*-acetyl-D-galactosamine.

We have retested the application of lectins for this purpose, and we observed that some of them had specific and strong agglutinins for the C group. The structure of their cell surface was also studied by use of inhibition reactions with simple sugars. Group C hemolytic streptococci can be the cause of scarlet fever (4) as well as group A and group G, so they should not be neglected from the clinical viewpoint.

The seeds we used were partly of commercial origin and partly collected by us in the field. Purified agglutinin and mitogen (7) from *Wisteria floribunda* seeds were kindly offered by T. Osawa (Faculty of Pharmaceutical Sciences, University of Tokyo).

Finely powdered seeds (1 g) were suspended in 10 ml of 0.9% NaCl solution and allowed to stand for 1 h at 37 C and overnight at 4 C with occasional stirring. Clear supernatant fluids were obtained by centrifugation.

In the case of *W. floribunda* (12), solid $(NH_4)_2SO_4$ was added to the supernatant fluid to give 40, 70, or 100% saturation. The fractions thus obtained were dialyzed against distilled water until free from NH_4^+, centrifuged, and lyophilized. This "crude mitogen" was further purified in the Faculty of Pharmacy, University of Tokyo, by chromatography successively on SE-Sephadex C-50 and Sepharose 6 B to give two fractions of strong hemagglutinin and mitogen.

Rabbit anti-A (J 17 A 4), -C (D3), -G (Valente), and -L (SHC16) sera were prepared according to Akiyama et al. (1) with the use of strains kindly offered by the World Health Organization International Reference Center for

Streptococcus Typing (Prague).

About 50 strains of each A group (20 strains of type 12, 10 of type 4, 5 of type 1, 5 of type 6, 5 of type 13, and 1 strain each of B 3264, type 3, type 9, and type 10), C group, and G group, as well as two strains of each D, E, F, L, M, N, O, and P groups, were digested with hog pancreatic extract by the method of Akiyama et al. (1).

One loopful (diameter, 1.0 mm) of antiserum or lectin was mixed well with one loopful of bacterial suspension on a glass slide, and reactions were read within 1 min.

For titration, twofold lectin dilutions in 0.9% NaCl solution were mixed with an equal volume of the bacterial suspension.

In inhibition tests with sugar, twofold serial dilutions of each sugar (0.1 ml) were added to an equal volume of lectin solution so diluted as to have a specific agglutinin titer of 1:8. The mixture was incubated for 1 h at room temperature and then overnight in a refrigerator. The bacterial suspension was added and the reaction was read.

For the absorption test with blood cells, 0.2 ml of human blood cells (O, A, B, or AB) was washed twice and added to an equal volume of lectin solution with a titer of 1:8. The mixture was incubated for 1 h at room temperature and then overnight in a refrigerator. The supernatant fluid obtained by centrifugation was tested for agglutinating activity on blood cells and bacteria. In another test, 0.2 ml of O, A, B, or AB cells was washed twice, mixed with an equal volume of undiluted lectin solution, and treated as just described. Twofold dilutions of the supernatant fluid were tested with the bacterial suspension.

We found that group C streptococci were agglutinated by anti-C serum, soybean, *W. floribunda* (ammonium sulfate fractions 40 to 70% and 70 to 100%), purified agglutinin, and purified mitogen. They were not agglutinated by the sera anti-A, anti-G, and anti-L nor by *Phaseolus vulgaris* or green peas.

Streptococci of groups A, B, D, E, F, G, H, L, M, N, O, and P were tested with the same reagents. Although types A, C, and L were agglutinated by the homologous sera, the other reactions were negative.

The 70 to 100% crude extract of *W. floribunda* acted on group C streptococci until dilutions as high as 1:10,000. This crude extract was separated into two homogeneous fractions—"agglutinin" and "mitogen"—by chromatography with Sephadex C-50 and Sepharose 6 B successively. The purified agglutinin acted on group C streptococci much more strongly (titer about 20,000) than the purified mitogen (titer 700).

Two of 50 strains of group C streptococci cross-reacted with anti-C and anti-G sera. These two strains had been identified as group C, giving a much stronger precipitation reaction with anti-C serum than with anti-G serum. However, they were not agglutinated at all by specific anti-C lectins.

Fractions of *W. floribunda* and soybean lectin were strongly inhibited in their agglutinating activity on group C streptococci by *N*-acetyl-D-galactosamine (Table 1).

Soybean lectin was slightly inhibited by galactose but not by lactose; inversely, lectin of *W. floribunda* was slightly inhibited by lactose but not by galactose. The following substances tested for inhibiting the two lectins were negative: L-fucose, D-sorbit, dulcit, L-sorbose, L-arabinose, raffinose, erythrit, glucose, maltose, mannose, xylose, isodulcit, and lobeaurose.

Contrary to our expectation, the agglutinin titers of the lectin solutions for group C streptococci were not diminished by absorption with O, A, B, or AB human blood cells.

Our results show that the extract of *W. floribunda* agglutinates group C streptococci and does not react with any other streptococcal serotype. This rapid and efficient method for identification of group C streptococci may be useful clinically. The test, which is simple and quite specific, could be adopted in any clinical diagnostic laboratory with ease.

By saving time and trouble, such as pretreatment of the bacteria for immunization, injection of bacteria into rabbits, and absorption of

TABLE 1. *Inhibition of agglutinating activity of lectins on group C streptococci by simple sugars*

Inhibiting substance	Minimal amt of substance giving inhibition (mg/ml)			
	Wisteria floribunda ammonium sulfate		Purified agglutinin	Soybean
	40–70%	70–100%		
N-acetyl-D-galactosamine	≤ 0.63	≤ 0.63	≤ 0.63	≤ 0.63
D-Galactose	>20	>20	>20	10
Lactose	10	10	10	>20

the antiserum to eliminate unspecific antibody, the introduction of protectin and lectins in the field of bacteriology contributes to the identification of group C streptococci. Comparative tests with *H. pomatia* protection and *W. floribunda* lectin are indicated to show which method is easier, cheaper, and quicker.

Considering the positive absorption test with soybean lectin and its inactivity against human blood cells, the operation sites for group C streptococci and human blood cells do not seem to be the same.

There are lectins inhibited either by galactose or by lactose in their agglutinating activity on group C streptococci, *N*-acetyl-D-galactosamine being the terminal unit of the serologically specific structure. This cell wall sugar is responsible for the reactions of the streptococci with their antiserum and with the lectins.

This investigation was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo and the Conselho Nacional de Pesquisas.

LITERATURE CITED

1. Akiyama, S., K. Takizawa, A. Matsushima, K. Nagashima, and Y. Miyamoto. 1967. Grouping of hemolytic streptococci by agglutination. Annu. Rep. Kanagawa Prefectural Institute of Hygienic Sciences **17**:1-3.
2. Bird, G. W. G. 1952. Relationship of the blood subgroups A_1, A_2 and A_1B, A_2B to hemagglutinin present in the seeds of *Dolichos biflorus*. Nature (London) **170**:674.
3. Boyd, W. C., and E. Shapleigh. 1954. Separation of individuals of any blood group into secretors and non-secretors by use of a plant agglutinin (lectin). Blood **9**:1195-1198.
4. Cayeux, P. 1973. Cours de microbiologie systematique, Chap. 32, p. 1, 6, 8. Institut Pasteur, Paris.
5. Köhler, W., and O. Prokop. 1967. Agglutination von Streptokokken der Gruppe C durch ein Agglutinin aus *Helix pomatia*. Z. Immunitaetsforsch. Allerg. Klin. Immunol. **133**:50-53.
6. Köhler, W., and O. Prokop. 1967. Agglutinationsversuche an Streptokokken mit dem Phytagglutinin von *Dolichos biflorus*. Z. Immunitaetsforsch. Allerg. Klin. Immunol. **133**:171-175.
7. Köhler, W., O. Prokop, and O. Kühnemund. 1973. Routine identification of group-C streptococci by means of an agglutinin (protectin) from the albumin gland of the edible snail, *Helix pomatia*. J. Med. Microbiol. **6**:127-130.
8. Landsteiner, K. 1920. Spezifische Serumreaktionen mit einfach zusammengesetzten Substanzen von bekannter Konstitution. Biochem. Z. **104**:280-299.
9. Morgan, W. T. J., and W. M. Watkins. 1953. The inhibition of the haemagglutinins in plant seeds by human blood group substances and simple sugars. Brit. J. Exp. Pathol. **34**:94-103.
10. Ottensooser, F., and K. Silberschmidt. 1953. Hemagglutinin anti-N in plant seeds. Nature (London) **172**:914.
11. Ottensooser, F., M. Sato, R. Sato, and H. Kurata. 1972. Lectin specificity induced by a fungus. Vox Sang. **22**:354-358.
12. Toyoshima, S., Y. Akiyama, K. Nakano, A. Tonomura, and T. Osawa. 1971. A phytomitogen from *Wisteria floribunda* seeds and its interaction with human peripheral lymphocytes. Biochemistry **10**:4457.
13. Watkins, W. M., and W. T. J. Morgan. 1952. Neutralization of the anti-H agglutinin in eel serum by simple sugars. Nature (London) **169**:825-826.