INCOMPLETE PARTIAL ANTIBODY (OF THE ANTI-B TYPE) OF AGGLUTINOID FROM THE SEEDS OF SOPHORA JAPONICA L.

M. I. Potanov

Scientific Research Institute of Forensic Medicine (Director, Honored Scientific Worker of the RSFSR, Professor V. I. Prozorovskii) USSR Ministry of Health, Moscow
(Presented by Active Member AMN SSSR N. N. Zhukov-Berezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 53, No. 5, pp. 98-102, May, 1962
Original article submitted June 20, 1961

In 1888, i.e., 13 years before Landsteiner's discovery of isohemagglutination [12], and 14 years before his preparation of anti-A and anti-B hetero-immune sera, Stillmark observed the agglutination of human erythrocytes by an extract of castor beans. Renkonen [17], and Boyd and Reguera [5], working independently found in many plants of the bean family the agglutinins (lectins, in Boyd's terminology) which in physiological saline reacted specifically with human erythrocytes containing one or other agglutinogen of the system ABO (H). In the USSR N. I. Blinov and P. N. Kosyakov [2] studied the problem of plant agglutinins.

Some authors [3, 4, 7, 8, 9, 11, 13] showed that extracts of certain seeds could agglutinate erythrocytes only if the latter were treated with enzymes, or if the reaction was carried out in a colloidal medium. These measures enhance the activity of the extracts containing "salt" hemagglutinins. It was therefore thought that special incomplete plant agglutinins must exist, although the evidence was not sufficiently complete to substantiate such a general conclusion.

Krüpe [9] advanced most convincing proof of the presence of incomplete antibodies in plants. He was able to produce agglutination of human erythrocytes of group A which had first been treated by an extract of seeds of Phaseolus limensis L. by treating them with rabbit serum containing antibodies against proteins of these seeds. Probably he had discovered an antibody of the cryptagglutinoid type.

In extracts from seeds of Sophora japonica L. (S.j.L) we have found an anti-B "salt" agglutinin which does not agglutinate human erythrocytes containing agglutinogens A, O, H, M, N, S, P, Le (A+), La(B+), Fy(A+) or Rh(C, D, E, c, e) either undiluted, or in various dilutions. When the extracts were titrated in physiological saline, zones in which there was a complete absence of agglutination were observed (from the undiluted extract to a dilution of 1 : 2-1 : 8). In the present investigation we have attempted to account for the presence of this zone and the nature of the inhibitor.

METHOD

S. j. L. seeds obtained from various botanical gardens in the USSR* were ground to a powder and 1 g of the flour was extracted with 10 ml of sterile physiological saline (for 2 hours at 37°, and then for 18-20 hours at 5°). The fluid was centrifuged, filtered through ashless filter paper, and kept at 5° without the addition of any antibacterial substances.

The ability of the extracts (either undiluted or in several dilutions) was studied in test tubes by bringing them in contact with a 2% suspension of standard erythrocytes (3 drops of fluid plus 1 drop of the suspension) and the centrifuging for 1 minute at 1000-1500 revs/minute. The results of the reaction were assessed by the naked eye, and microscopically.

RESULTS

Starting from the idea that the phenomenon of the zone is due to the presence in the extracts of incomplete phytagglutinins possessing the property of blocking erythrocytes, we carried out a number of tests similar to those

*The Sochi arboretum, gardens of Tashkent, Ashkhabad, Alma-Ata, Dyshanbe, and the Kyban experimental station of the All-Union Plant Growing Institute.

which have been worked out for the identification of incomplete agglutinins in serum, i.e.,: 1) a test with blocking; 2) a direct Coombes test; 3) the reaction of conglutination in a colloidal medium, using the method of Fisk and McGee [4]; 4) the reaction of agglutination of erythrocytes treated with trypsin.

In the test for blocking, erythrocytes of group B, which had not been agglutinated after a 1½ hour contact with an undiluted S.j.L. extract (over the range from 4 to 37°) were exposed to the repeated action of the same extract diluted 1 : 32-1 : 64, which is optimal for agglutination. Erythrocytes treated this way lost the ability to be agglutinated by extracts either of S.j.L. seeds or of the seeds of Coronilla varia L. (C.v.L.), which also contained specific anti-B lactin. Experiments on the use in crossed reactions of extracts of S.j.L. seeds grown in various places showed that erythrocytes in which agglutinogen B was blocked by an undiluted extract of the seeds obtained from one place (for example Sochi) were not agglutinated by extracts of seeds originating elsewhere (Ashkhabad, Tashkent, Alma-Ata, and other places) and diluted to the active condition. Extracts of S.j.L. caused no blockade of erythrocytes of group O and A with respect to goat heteroimmune serum anti-O(h), to human isoagglutinating serum α, or to rabbit anti-A heteroimmune serum.

Blocking Test

Undiluted extract of S.j.L. (Sochi) plus a 2% suspension of group B erythrocytes							
Low agglutination of erythrocytes							
The following reagents							
Anti-B phytoagglutinins					Sera		
S.j.L. (1 : 64)					C.v.L. (1 : 8) Nikitskii Garden (Crimea)	Hetero-immune anti-B rabbit	Isoserum β
Sochi	Ashkhabad	Dushanbe	Tashkent	Alma-Ata			
Hemagglutination reaction							
—	—	—	—	—	—	+++	+++
Control (untreated erythrocytes)							
+++	+++	+++	+++	+++	+++	+++	+++

However, blockade of the erythrocytes of group B did not prevent their agglutination as a result of the subsequent action of isoagglutinating sera β and rabbit heteroimmune anti-B sera (see table).

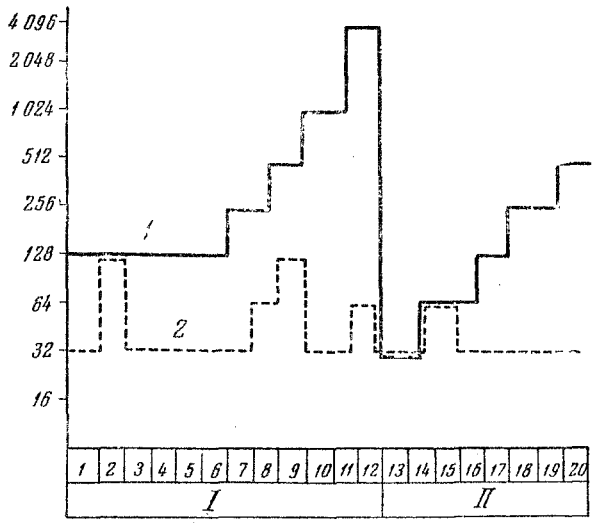
In the direct Coombes test Krüpe used anti-seed protein, but we used the normal antiglobulin serum, and invariably obtained a positive reaction with group B erythrocytes, and it was always negative with group O erythrocytes. With group A erythrocytes there was a very weak reaction, occurring 8-18 minutes later than that of the group B erythrocytes.

The Fisk and McGee test [6] was strongly positive with erythrocytes of group B, and negative with those of group O. The weakly shown agglutination of group A erythrocytes rapidly passed off.

The agglutination reaction with trypsin-treated erythrocytes showed that the zone of delay is continuous with a zone of weak agglutination; erythrocytes of group O and group A begin to be agglutinated by the extract, those of group A showing a delay zone. The blocking action occurs, (but in a very reduced form) and it takes place also when the extract is titrated with human group AB serum. Under these conditions the extract loses its specificity. The delay zone of the agglutination may be readily eliminated by dilution of the extract with physiological saline, or by a single absorption with an equal volume of group B erythrocytes (but not group A or group O erythrocytes). The inhibitor of the agglutination combines very stably with group B erythrocytes: it is not eluted when the erythrocytes are warmed to 56°; if the blocked erythrocytes are washed 10 times with physiological saline, when they are treated an extract diluted to the active condition they form small clumps which are visible only under the microscope.

Heating the extract in a water bath at 90° for one hour eliminates the "salt" agglutinin, but does not inactivate the inhibitor of the agglutination. The reaction of the delay of agglutination, during which, after contact with various dilutions of the heated extract, the group B erythrocytes were exposed to the action of active unheated extract (1 : 32-1 : 64) showed that the titer of the inhibitor, (masked by agglutinin) was considerably greater than could be expected from the delay zone. Thus, the titer of the agglutinin with respect to erythrocytes of a particular person was 1 : 8192, but the action of the inhibitor appeared at a dilution of the extract as low as 1 : 256.

It was also shown that the complete anti-B agglutinin (S.j.L.) reacts more strongly at 5° than at 20 or 37°, and that its activity increases on titration in serum of group AB and with trypsinized erythrocytes, and that it is absorbed by group B erythrocytes, and is eluted from them. Erythrocytes from 20 subjects (12 of group B, 8 of group AB) showed great differences (up to 128 times) in the extent to which they were agglutinated by anti-B lectin, though they were comparatively uniform with respect to isoserum β (a difference of up to 4 times, see figure).



Agglutination of erythrocytes of groups B (I) and AB (II) by (1) anti-B lectin and (2) by isoserum β. Isoserum β was diluted three times, and anti-B lectin was not diluted.

Besides erythrocytes of groups B and AB in man, complete lectin agglutinates rabbit erythrocytes (and there is a delay zone) but does not agglutinate erythrocytes of the chick, rat, guinea pig cat, dog, pig, goat, boar, ox, or horse. With rabbit erythrocytes blocked by lectin, a positive Coombes test was obtained (it was negative with guinea pigerythrocytes).

A 1 : 5 S.j.L. extract also contains precipitins for human serum and for the sera of the chick, rabbit, dog, pig, boar, ox, elk, and horse. These precipitins have a titer of 1 : 4-1 : 8, and have no selective action. Experiments on the precipitation by lectin of extracts from the saliva of subjects who were "excretors" of substances H, A, and B were negative.

The results of the experiments confirmed the correctness of the working hypothesis that the inhibitor of agglutination in S.j.L. extracts is an incomplete antibody (incomplete lectin). This antibody which is active between 5 and 37° is thermostable, and cannot be eluted; it belongs to the agglutinoids, because it possesses the property of blocking trypsinized erythrocytes in saline solution, and to a lesser extent in a colloidal medium. It is revealed in the indirect Coombes, and in the Fisk and McGee tests.

S.j.L. extracts contain a mixture of complete and incomplete lectins of the same specificity. Because the extract agglutinates rabbit erythrocytes which are known to contain antigens B_2 and B_3, and is not active with respect toguinea pig erythrocytes, which contain antigen B_3, and because the agglutination curves of erythrocytes of groups B and AB with respect to lectin and isoserum β are completely different, we have reason to suppose that complete lectin is the partial agglutinin anti-B_2, or is closely related to it in its properties. The further evidence from the positive Coombes test with rabbit erythrocytes and the negative test with guinea pig erythrocytes indicates that the agglutinoid also has the corresponding specificity (anti-B_2).

The results we have obtained contradict the notion that the inhibitor of the agglutination is made up of sugars capable of inactivating plant agglutinins [8, 10, 14, 15].

In a study of erythrocytes of groups B and AB with lectin anti-B (S.j.L.) the effectiveness of the partial agglutinin B varies greatly from one person to another, although, as is known, this variation does not occur when isoserum β or heteroimmune rabbit anti-B serum is used. This difference in the agglutinogen B is so great that it approaches the variety of the forms of agglutinogen A.

The evidence of the presence in plants not only of complete but also of incomplete antibodies possessing the properties of serum agglutinins supports the concept put forward by N. N. Zhukov-Vereshnikov [1] of the primary immunologic reactivity of living matter.

We have been unable to find any reference to the occurrence in human or animal serum of an incomplete partial antibody to human B agglutinogen. The discovery of a partial anti-B agglutinoid of vegetable origin to some extent fills a gap, and may be used in immunological and serological investigations for a further study of the antigenic structure of the human and animal organisms.

SUMMARY

The antibody which we have revealed possesses the property of blocking human type B erythrocytes in physiological saline; its ability to act in this way on trypsinized erythrocytes is reduced in colloidal solution; it is detectable in the Coombes as well as in the Fisk and McGee test, it is active over a range from 5 to 37°, and is thermostable, and cannot be eluted. The antibody is evidently specific to the partial B_2 agglutinogen.

LITERATURE CITED

1. N. N. Zhukov-Verezhnikov and M. I. Sokolov, In book: The prophylaxis of infections by living vaccines. [in Russian] Moscow, p. 14 (1960).
2. P. N. Kosyakov, Antigenic substances of the organism and their significance in biology and medicine. Moscow, p. 175 (1954).
3. G. W. Bird, Nature, Vol. 174, p. 1015 (1954).
4. Idem, Brit. med. Bull., Vol. 15, p. 165 (1959).
5. W. C. Boyd and R. M. Reguera, J. Immunol., Vol. 62, p. 333 (1949).
6. R. T. Fisk and C. A. McGee, Am. J. clin. Path., Vol. 17, p. 737 (1947).
7. P. Herzog, Csl. Biol., Vol. 7, p. 444 (1958).
8. Y. Kimura and T. Nagano, Japan, J. legal. Med., Vol. 13, p. 562 (1959).
9. M. Krüpe, Blutgruppenspezifische Pflanzliche Eiweibkorper (Phytagglutinine). Stuttgart (1956).
10. M. Krüpe and W. Dötzer, Ann. Med. exp. Fenn., Vol. 35, p. 1 (1957).
11. M. Krüpe and A. Ensgraber, Planta, Bd. 50, S. 371 (1958).
12. K. Landsteiner, Wien. klin. Wschr., Bd. 14, S. 1132 (1901).
13. O. Mäkelä and P. Mäkelä, Ann. Med. exp. Fenn., Vol. 34, p. 402 (1956).
14. O. Mäkelä, Ibid., Vol. 35, Suppl. 11, p. 133 (1957).
15. W. T. Morgan and W. M. Watkins, Brit. J. exp. Path., Vol. 34, p. 94 (1953).
16. F. Ottensooser and K. Silberschmidt, Nature, Vol. 172, p. 914 (1953).
17. K. O. Renkonen, Ann. Med. exp. Fenn., Vol. 26, p. 66 (1948).
18. H. Stillmark, Ueber Ricin; ein giftiges Ferment aus den Samen von Ricinus comm. L. und einigen anderen Euphorbiaceen. Schnakenburg (1888).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
