On Hemagglutinin in Antipneumococcal Type XIV Horse Serum*

By

Hajime Masamune and **Zensaku Yosizawa**

(正宗 一)

(吉澤 善作)

From the Medico-chemical Institute (Director:

Prof. H. Masamune)

and

Tosiyuki Miki

(三木 敏行)

From the Forensic Medical Institute (Director: Prof. T. Murakami),

Tohoku University, Sendai

(Received for publication, March 8, 1954)

In 1938 Finland & Curren¹⁾ discovered the occurrence of agglutination of human red cells of all four blood groups by type XIV antipneumococcal horse serum, and in the following year, Goebel *et al.*²⁾ and Weil & Sherman³⁾ worked on this subject. Goebel & others' results suggested that the type XIV specific polysaccharide (SXIV) contains, in addition to the type specific structure, a structure in common with the Group A mucopolysaccharide which precipitated more than half of the total antibody in the serum reacting with SXIV. In the experiments of Weil & Sherman, the hemagglutinating factor of the serum was completely absorbed by the type-specific carbohydrate of type XIV and on the other hand, the serum agglutinated not only human erythrocytes and stromata but also rabbit and cat erythrocytes. Furthermore, human organs (heart, kidney and liver) absorbed the hemagglutinating quality from the serum.

Later on Kabat made with his co-workers⁴⁾⁻⁸⁾ repeated studies of that hemagglutination in connection with the structural relationship between the blood group substances within the ABO classification and the type XIV pneumococcus polysaccharide. These authors found that all of A, B and O human and hog blood group mucopolysaccharides cross-reacted with the antiserum and that the cross-reactivity was increased by controlled acid hydrolysis of the group substances. Since SXIV consists of acetylglucosamine and galactose²⁾ and the group mucopolysaccharides contain L-fucose

* Read before the 2nd Meeting of the Japanese Union of Physiological Sciences, Tokyo, November 10, 1952.

in addition to those sugar components, Kabat put forward the opinion that a series of N-acetylglucosamine-galactose residues, possessing at various points partial structures similar to those in the chain of SXIV, forms the main chain of the group substances attached with L-fucose molecules projecting outward at various points. According to him, the L-fucose residues prevent close approach of the antibody molecules to the reacting groupings on the main chain so that removal of them by the partial hydrolysis provides cross-reacting sites. By the hydrolysis the group substances lost the specific activity and therefore he said that "the labile amino acid", which is split off thereby," may possibly be responsible for conferring blood group activity on the galactose-glucosamine polysaccharide, since the fucose (or at least some of it) is not necessary for blood group activity."

More recently, Annison & Morgan⁹⁾ considered more probable that the agglutination of human erythrocytes by the antiserum is due to components of the red cell surface other than A, B and H(O) receptors such as the product of the Le^a gene. The reason was that the group substances of ABO type obtained from human ovarian cysts failed to react with the antibody but the Le^a substance from the same source did react. They thought the latter to be a material containing a carbohydrate possibly with basic glucosamine-galactose structure, which according to Kabat is responsible for the cross-reactivity mentioned, being based on the indirect evidence afforded by a periodate oxidation experiment on erythrocyte surface. The Group A and H substances from ovarian cysts, which did not precipitate with the antiserum, gave the reaction after mild acid hydrolysis causing scission of L-fucose¹⁴⁾¹⁵⁾ from the carbohydrate moiety. The authors assumed the A and H group substances above to be similar to those in red cells, despite that the group substances in erythrocytes are compound lipids¹¹⁾⁻¹³⁾ containing hexosamine and hexose but no L-fucose.

We undertook to scrutinize those studies with a horse serum against antipneumococcus type XIV (Kabat's H-618) which Prof. Kabat kindly supplied.¹⁶⁾ At first, precipitin test of the serum was tried with a Group A substance obtained after alkaline cleavage at 20°C of pig stomach mucus and its products after heating with acetic acid, whose preparation procedures and analyses have been already reported (Chs. I, II and III in an article of Masamune & Yosizawa¹⁷⁾). As is shown in Table I, no positive precipitin reaction occurred with any of Chs. I, II and III which were Group A active up to dilutions of $1:6.4 \times 10^5$, $1:4 \times 10^4$ and $1:4 \times 10^4$ respectively and Group O active (against normal anti-O goat serum diluted to titre 4) up to dilutions of $1:6.4 \times 10^4$, $1:8 \times 10^3$ and $1:10^3$ respectively. The preparations contained glucosamine, galactose and L-fucose, the methylpentose content decreasing from left to right in the order arranged that Ch. III contained only 0.8% fucose. Even Ch. I was a scission product

of a natural group substance and the alkali-labile fragments split off during preparation (Cf. Masamune, Yosizawa and Miki¹⁸⁾) may have a structure reacting with the hemagglutinating antibody, nevertheless, the finding above furnishes at least an evidence that configurations not related to the type XIV pneumococcus polysaccharide can develop group action.

TABLE I

Precipitin Test of the Type XIV Antipneumococcal
Horse Serum with Chs. I, II and III

The ring test was applied. The results of the test were judged after 5 hours from the overlaying. — : negative reaction. Ch. I: a group mucopolysaccharide separated from pig stomach mucus by scission with 1 *N* NaOH at 20°C. Chs. II and III: products from Ch. I obtained after heating at 100°C with 1 *N* CH₃COOH for 1 and 3 hours respectively.

Ch.	Dilution of the antigens (1 :)					
	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶	10 ⁷
I	—	—	—	—	—	—
II	—	—	—	—	—	—
III	—	—	—	—	—	—

In the next, absorption of the horse serum was processed with type XIV pneumococcus, erythrocytes and saliva of various groups separately, followed by determination of agglutinin titre for erythrocytes. The results will be described item by item.

TABLE II

Occurrence of Hemagglutination by the Type XIV Antipneumococcal Horse Serum treated with Type XIV Pneumococcus

Various amounts of type XIV pneumococcus were suspended in 1 cc. of physiological saline and 1 cc. portions of the serum diluted 2.5-fold were absorbed with the suspensions. The serum portions absorbed were progressively doubled with physiological saline and 1 drop was transferred from each of them onto a glass slide, followed by addition of 1 drop of a 1% erythrocyte suspension. The mixtures were occasionally shaken at room temperature and the results were judged after 30 minutes. The figures in the table express agglutinin titres.

Erythrocytes	Unabsorbed serum	Amount of the bacteria taken for absorption (mg.)		
		2	5	8
Ae (1)	160	40	10	no aggl. at 5-fold dilution
BE (1)	320	40	10	"
O (1)	320	40	10	"

1) The agglutinin titres of the antiserum toward various groups of erythrocytes lowered to equal values stepwise by absorption with increasing amounts of the pneumococci, whose lyophile¹⁶⁾ was also given from Prof. Kabat (Table II). Although the titre of the serum unabsorbed was somewhat lower toward A erythrocytes than toward the others, the consequence allows the possibility that the agglutination of the different groups of erythrocytes is caused by one and the same agglutinin.

2) AS, BS, OS and Os salivas gave no positive precipitin reaction with the serum (Table III), but all of them absorbed the hemagglutinin

TABLE III

Precipitin Test of the Type XIV Antipneumococcal Horse Serum as such with Salivas of Individuals belonging to Different Groups

The procedure of the test was the same as that in Table I. — : negative reaction.

Saliva	Dilution of salivas (1:)									
	1	3	10	$\frac{3 \times}{10}$	10^2	$\frac{3 \times}{10^2}$	10^3	$\frac{3 \times}{10^3}$	10^4	∞
OS (1)	—	—	—	—	—	—	—	—	—	—
Os (1)	—	—	—	—	—	—	—	—	—	—
AS (1)	—	—	—	—	—	—	—	—	—	—
As (1)	—	—	—	—	—	—	—	—	—	—
BS (1)	—	—	—	—	—	—	—	—	—	—
Bs (1)	—	—	—	—	—	—	—	—	—	—

(Tested with O erythrocytes) (Table IVA) notwithstanding that the secretor salivas only absorbed O erythrocyte agglutinin from an eel serum (Table IVB). Thus the agglutinin in the horse serum was found removable by a factor or factors other than the group substances which are owned by A, B and O salivas, that have a structure which, judging from the present findings together with those of Weil and Sherman, is common to human, rabbit and cat.

3) With the conclusion above do not conflict following results which resembled those of the predecessors. As seen in Table V, erythrocytes belonging to a particular group decreased the hemagglutinin potency for various erythrocytes to similar extents, regardless of their blood group and strength of E potency.

In short, it might be claimed that the hemagglutinin in question is produced by the XIV pneumococcus polysaccharide and combines with carbohydrate-containing substances, which have a configuration common to human, rabbit and cat but have nothing to do with group activity.

TABLE IV

Occurrence of Agglutination of Erythrocytes by the Type XIV Antipneumococcal Horse Serum and an Eel Serum both after Treatment with Salivas of Various Groups

To 0.2 cc. portions of each saliva, doubled progressively with physiological saline, was added 0.2 cc. of one of the antisera diluted to agglutinin titre 8. The mixtures were stood at 37°C and at room temperature for 1 hour each and in a refrigerator (0°C) overnight in turn. Then 1 drop was transferred from each of the mixtures onto a glass slide to examine the hemagglutinating potency of the serum as in the experiment of Table II. O erythrocytes were used in the test here. — : no agglutination, + : slight aggl., ++ : not-marked aggl.

Saliva used for absorption	Dilution of salivas for absorption (1 :)					
	1	2	4	8	16	32
A. Hemagglutination by the horse serum absorbed						
OS (2)	—	—	+	+	+	
Os (1)	—	+	+	+	+	
AS (2)	—	+	+	+	+	
BS (2)	—	+	+	+	+	
B. Hemagglutination by the eel serum absorbed						
OS (2)	—	—	—	—	—	+
Os (1)	+	+	+	+	+	++
AS (2)	—	—	—	—	+	+
BS (2)	—	—	—	—	+	+

SUMMARY

1. A specimen of Group A substance (contaminated with some Group O substance), obtained after alkaline scission at 20°C of pig stomach mucus, as well as the products from it after conditioned acid hydrolysis did not precipitate with an antipneumococcus type XIV horse serum.

2. The horse serum lost the agglutinating potency toward various groups of erythrocytes to equal extents when absorbed with type XIV pneumococcus.

3. The hemagglutinin in the horse serum was absorbed by secretor and non-secretor salivas in similar grades.

4. The horse serum treated with erythrocytes of one blood group lost the potency of agglutinating various groups of erythrocytes in similar degrees.

We were led from the findings above and those of former investigators to the conclusion that the hemagglutinin in the type XIV antipneumococcal

TABLE V

Hemagglutination by the Type XIV Antipneumococcal Horse
Serum treated with Erythrocytes of Various Groups

The examination was processed in a similar manner as described in Table
II. The figures express agglutinin titres.

A. The serum was absorbed with AB erythrocytes

Here the serum was diluted 5-fold prior to absorption.

Erythrocytes* for determining aggl. titre	Before absorption	Group and volume of erythrocytes used for absorption			
		ABe (1)		ABE (2)	
		1/5 volume	additional 1/5 vol.	1/5 vol.	add. 1/5 vol.
ABE (1)	320			20	no aggl. at 5-fold dilution
ABe (1)	320	20	no aggl. at 5-fold dilution	20	"
ABE (2)	160			10	"
ABE (3)	320			20	"
ABe (2)	160	10	"	10	"
O (1)	320	20	faint aggl. at 5- fold dilution	20	faint aggl. at 5- fold dilution

* The AB erythrocyte samples in the arranged order were agglutinated by an eel serum at dilutions of 1:200, 1:10, 1:200, 1:200 and 1:<10 respectively.

B. The serum was absorbed with O, A or B erythrocytes

The serum was absorbed without prior dilution.

Erythrocytes for measuring aggl. titre	Before absorption	Group and volume of erythrocytes used for absorption					
		O*		A*		B*	
		1/2 volume	additional 1/10 vol.	1/2 vol.	add. 1/10 vol.	1/2 vol.	add. 1/10 vol.
Ae (1)	160	4	no aggl.	4	no aggl.	4	no aggl.
BE (1)	320	4	"	4	"	4	"
O (1)	320	4	"	8	"	8	"

* Collected from several persons.

horse serum reacts with carbohydrate-containing factors other than the A, B and O group substances with a surface structure common to species human, rabbit and cat.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

References and Notes

- 1) Finland & Curren, Science, 1938, **87**, 417.

- 2) Goebel, Beeson & Hoagland, J. Biol. Chem., 1939, **129**, 455.
 - 3) Weil & Sherman, J. Immunol., 1939, **36**, 139.
 - 4) Kabat, Bendich, Bezer & Knaub, J. Exp. Med., 1948, **87**, 295.
 - 5) Kabat, Baer, Bezer & Knaub, *ibid.*, 1948, **88**, 43.
 - 6) Baer, Dische & Kabat, *ibid.*, 1948, **88**, 59.
 - 7) Kabat, Bact. Rev., 1949, **13**, 189.
 - 8) Kabat, Baer, Day & Knaub, J. Exp. Med., 1950, **91**, 433.
 - 9) Annison & Morgan, Bioch. J., 1952, **50**, 460.
 - 10) Morgan & Watkins, Brit. J. Exp. Path., 1951, **32**, 34.
 - 11) Hamasato, Tohoku J. Exp. Med., 1950, **52**, 17. Cf. also Masamune and co-workers' papers on group lipids in the same Journal.
 - 12) Yamakawa & Suzuki, J. Bioch., 1952, **39**, 393.
 - 13) Yamakawa, Matsumoto, Suzuki, Iida and Kuniyuki, Seikagaku (Japanese J. for Jap. Bioch. Soc.), 1954, **26**, 398.
 - 14) Aminoff, Morgan & Watkins, Bioch. J., 1950, **46**, 426.
 - 15) Annison & Morgan, *ibid.*, 1952, **52**, 247.
 - 16) We express cordial gratitude to Prof. Kabat for the gift.
 - 17) Masamune & Yosizawa, Tohoku J. Exp. Med., 1954, **60**, 135.
 - 18) Masamune, Yosizawa & Miki, *ibid.*, 1954, **60**, 147.
-