120. Studies on Haptoglobin. VIII

*Effect of Haptoglobin on Immunogenicity of Hemoglobin**

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Introduction. In the course of the studies on the binding site of human adult hemoglobin (Hb A) for haptoglobin (Hp), we suggested that antigenic determinants of Hb A are not modified nor masked by complex formation with Hp, and, moreover, there is no evidence for the appearance of new antigenic determinants in Hb A on complex formation with Hp.¹⁾ Although there is no difference in quality of antigenic determinants of Hb A between free Hb A and Hb A associated with Hp, the reactivity of Hb A associated with Hp with anti-Hb A serum is stronger than that of free Hb A.¹⁾

In this communication, it was examined whether or not there is difference in inducibility of antibody against human Hb A in rabbits between free Hb A and Hb A associated with rabbit Hp.

Materials and methods. Preparation procedures for Hb A, hemoglobin A-rabbit haptoglobin complex (Hb A-Hp^{rab} complex), and hemoglobin A-human haptoglobin complex (Hb A-Hp^{hum} complex) were described elsewhere.²⁾

White adult rabbits were immunized with Hb A or Hb A-Hp^{rab} complex. One and a half mg as Hb A of antigen in a volume of 1.0 ml, mixed with an equal volume of Freund's complete adjuvant, was injected subcutaneously into rabbit's back. The same injection was repeated two weeks later. On the 14th, 21st, and 28th day after the primary immunization, serum was obtained from an ear vein for antiserum titration.

Serum dilution test was carried out according to the method of Martin.³⁾ Serum was diluted with 0.9% NaCl solution containing 1.5% gum arabic according to two-fold dilution method. Ring test was carried out in the 3×50 mm tubes and precipitation reaction was observed for 5 hours at room temperature. Number of titer shows the highest dilution at which a precipitation reaction could be still observed.

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Results and discussion. In the previous communication,¹⁾ we reported that there is a considerable difference in reactivity between free Hb A and Hb A-Hp^{rab} complex with anti-Hb A sera and anti-(Hb A-Hp^{rab} complex) sera, whereas as antigens there are not any qualitative differences between Hb A and Hb A-Hp^{rab} complex in rabbits. Although the mechanism of this phenomenon is not yet fully understood, for the comparison of immunogenicity, it is necessary to titrate the anti-(Hb A-Hp^{rab} complex) sera with free Hb A with which the anti-Hb A sera are titrated. Moreover, since the reactivity of Hb A-Hp^{hum} complex with anti-Hb A sera and anti-(Hb A-Hp^{rab} complex) sera is highest among three test antigens (Hb A, Hb A-Hp^{rab} complex, and Hb A-Hp^{hum} complex), and some anti-Hb A serum shows the precipitation reaction with Hb A-Hp^{hum} complex but not with free Hb A, titers of anti-Hb A sera and anti-(Hb A-Hp^{rab} complex) sera were determined with both free Hb A and Hb A-Hp^{hum} complex.

A direct comparison of immunogenicity between Hb A and Hb A-Hp^{rab} complex may be possible, because rabbit Hp associated with Hb A is expected to be not antigenic in rabbits. As shown in Table I,

Table I. Inducibility of antibody in rabbits of free Hb A and Hb A-Hp^{rab} complex. Antiserum was titrated with free Hb A and Hb A-Hp^{hum} complex

No. of rabbits	Stimulating antigen	Test antigen					
		Hb A			Hb A-Hp ^{hum}		
		14 ^a	21	28	14	21	28
111	Hb A 1.5 mg	0 ^b	8	8	0	32	16
112		0	4	8	0	32	16
113		0	2	0	0	16	4
114		0	0	0	0	8	4
115		0	1	0	0	8	4
121	Hb A-Hp ^{rab} 1.5 mg	0	16	16	4	64	64
122		0	16	16	0	32	32
123		0	8	8	0	32	16
124		0	16	16	4	32	32
125		0	0	0	0	4	1

a: Days after the primary immunization.

b: Number of titer shows the highest dilution at which a precipitation reaction could be still observed.

with respect to evoking antibody against Hb A in rabbits, Hb A associated with rabbit Hp *in vitro* is more effective than free Hb A. In four of five rabbits on the 21st and 28th day after the primary immunization, Hb A-Hp^{rab} complex induced the formation of antibody which was detected with free Hb A as a test antigen, and moreover

in two of five rabbits on the 14th day, the formation of antibody detected with Hb A-Hp^{hum} complex as a test antigen. On the other hand, free Hb A evoked in four of five rabbits the formation of antibody detected with free Hb A on the 21st day after the primary immunization, but on the 28th day only two of five rabbits maintained the level of antibody detected with free Hb A. On the 14th day, none of rabbit sera stimulated with free Hb A was found even with Hb A-Hp^{hum} complex to contain antibody against Hb A.

In general, the level of antisera from rabbits immunized with Hb A-Hp^{rab} complex is higher than that from rabbits immunized with free Hb A. Only an exception was rabbit No. 125 which responded very weak to the stimulating antigen (Hb A-Hp^{rab} complex).

It was further confirmed that antisera from rabbits immunized with free Hb A or Hb A-Hp^{rab} complex did not react with free human Hp.

It was apparent from the observation mentioned above that immunogenicity of Hb A is strengthened by complex formation with rabbit Hp *in vitro*. The presence of the remarkable difference in inducibility between free Hb A and Hb A associated with rabbit Hp seems to indicate that subcutaneously injected Hb A may not be bound with rabbit Hp *in vivo* prior to incorporation by macrophages.

In conclusion, there is difference in inducibility of antibody against Hb A in rabbits between free Hb A and Hb A-Hp^{rab} complex, although there is no difference in quality of antigenic determinants of Hb A between them.¹⁾

In attempt to understand the marked enhancement of immunogenicity of Hb A associated with rabbit Hp, susceptibility of free Hb A and Hb A associated with Hp to proteases was compared, and some possible mechanisms which account for this phenomenon will be discussed in the following communication.⁴⁾

Summary. With respect to evoking antibody against hemoglobin A in rabbits, immunogenicity of hemoglobin associated with rabbit haptoglobin *in vitro* is stronger than that of free hemoglobin. There is quantitative difference in inducibility of antibody against hemoglobin in rabbits between free hemoglobin and hemoglobin associated with rabbit haptoglobin, although there is no difference in quality of antigenic determinants of hemoglobin between them.

References

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