BRIEF NOTE

Serological and Immunological Differences between Two Hemagglutinins of *Haemophilus* *paragallinarum* Strain 221

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Hemagglutination by *Haemophilus paragallinarum* (Hpg) was first described by Kato et al. [4]. The hemagglutination-inhibition (HI) antibody response of chickens to Hpg vaccination was shown to be correlated with protection from Hpg infection [5, 6]. In Japan, the HI test has widely been used for evaluation of Hpg vaccine. In their previous paper, the authors showed that Hpg strain 221 possessed two hemagglutinins (HA) termed type 1 HA and type 2 HA, respectively, and that they were different in physicochemical properties and hemagglutinating activity [7]. It must be important to determine whether there are serological and immunological differences between them or not.

This paper deals with the serological and immunological differences between the two HA antigens in Hpg in order to clarify the properties of them further.

The two HA antigens were prepared in such manner as mentioned in the previous paper [7]. The HI test was conducted in the following manner: From chicken antiserum were prepared serial two-fold dilutions in 0.2 ml of phosphate buffer saline (PBS). Each dilution was mixed with an equal volume of each HA antigen contain-

ing 4 HA units in a test tube. To this mixture was added 0.4 ml of 0.5% chicken red blood cell suspension in PBS 10 min later. HI titer was expressed as the reciprocal of the highest dilution of antiserum that showed complete inhibition.

The slide agglutination (AGG) test was performed by the method of Iritani et al. [3]. The chickens used were 6-8 weeks old and derived from the LL line of the White Leghorn breed free from specific pathogens. They were inoculated intravenously with each killed HA antigen which had been adjusted to $10\times$ the density of 0.4 with absorbance at $540\text{ m}\mu$ in PBS. After 3 weeks, sera were collected from the immunized chickens.

The adsorption test was conducted in the following manner. Antiserum was adsorbed with the two HA antigens, which were then removed by centrifugation. The resultant HI titer of absorbed antiserum was determined by these antigens. Three weeks after inoculation, the chickens were challenged intranasally with 0.5 ml of viable Hpg cells containing 10^8 colony forming units/ml. They were examined for such clinical signs as nasal discharge and facial swelling and necropsied one week after

Table 1. Serological response and protective effect of two hemagglutinins of *Haemophilus paragallinarum* (Hpg)

Inoculation with	Geometric mean HI titer to		Geometric mean agg. titer	Clinical sign	Lesion	Recovery of Hpg
	Type 1 HA	Type 2 HA				
Type 1 HA antigen	1:46 (1:20-1:80) *	1:28 (1:20-1:40)	1:52 (1:20-1:80)	0/10**	0/10	0/10
Type 2 HA antigen	<1:5 (<1:5)	1:66 (1:20-1:80)	1:35 (1:5-1:80)	10/10	10/10	10/10
Control	<1:5 (<1:5)	<1:5 (<1:5)	<1:5 (<1:5)	10/10	10/10	10/10

* Minimum to maximum titers.

** No. of positive chickens/No. of chickens tested.

Table 2. Adsorption test between two hemagglutinins of *Haemophilus paragallinarum*

Antiserum to	Chicken No.	HI titer					
		Before adsorption		After adsorption with type 1 HA		After adsorption with type 2 HA	
		Type 1 HA	Type 2 HA	Type 1 HA	Type 2 HA	Type 1 HA	Type 2 HA
Type 1 HA antigen	1	1:80	1:40	<1:5	1:40	1:40	<1:5
	2	1:20	1:20	<1:5	1:20	1:20	<1:5
	3	1:40	1:20	<1:5	1:10	1:40	<1:5
	4	1:40	1:20	<1:5	1:20	1:40	<1:5
Type 2 HA antigen	5	<1:5	1:40	<1:5	1:20	<1:5	<1:5
	6	<1:5	1:20	<1:5	1:20	<1:5	<1:5

challenge.

The serological response and protective effect of the two HA antigens are shown in Table 1. It was revealed that type 2 HA antigen could not produce type 1 HA antibody in chickens, and that type 1 HA antigen produced not only type 1 HA antibody but also type 2 HA antibody. In contrast to the HI response, there was no significant difference in AGG response between the two HA antigens.

In the challenge test, protection was found not in the chickens inoculated with type 2 HA antigen, but in those inoculated with type 1 HA antigen.

Although the chickens immunized with type 1 HA antigen produced HI antibodies against both HAs, no cross-reaction was observed between type 1 HA and type 2 HA in the adsorption test (Table 2).

By serological response and adsorption test, the serological difference between the two HAs in Hpg cells was clarified. This difference may have been derived from the difference in physicochemical properties between two HAs reported previously. These results suggest that the Hpg cell may originally have two HAs. It is probable that type 2 HA antigen may exist in a more inner part of the Hpg cell than type 1 HA antigen. In fact, the authors pointed out previously that type 2 HA activity had appeared after proteolytic enzyme treatment [7]. Iritani [1] reported that trypsin-treated bacterial cells had lost some surface components, including type 1 HA.

The challenge test revealed that an immunological difference existed between the two HAs in Hpg cells. It was suggested that type 1 HA might consist of protein and

carbohydrate, but nothing was known about the composition of type 2 HA of Hpg cells [7]. Moreover, HG-HA which is composed of protein and carbohydrate seems to be a protective component of the Hpg cell [2]. It is understandable that type 2 HA is not protective antigen in Hpg strain 221.

In Japan, the HI test has been used as a means of determination of the immunizing potency of Hpg vaccine, although two HA antigens were known to exist in cells of Hpg strain 221. The results of the present investigation show that the HI test with type 2 HA antigen was not suitable for the evaluation of protective effect. Now, it is necessary to determine whether the HA

antigen used for the HI test is type 1 or type 2.

References

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要 約

ヘモフィルス・パラガリナルム 221 株における 2 種赤血球凝集素の血清および免疫学的相異 (短報): 山口 猛・岩城秀治・入谷好一 (塩野義製薬株式会社油日ラボラトリーズ)——ヘモフィルス・パラガリナルム 221 株中に存在する 2 種類の赤血球凝集素 (HA) は, それらの抗血清での赤血球凝集抑制反応および吸収試験によって明らかに区別された。更に, 感染試験において, タイプ 1HA 活性を示す抗原は感染防御性を有していたが, タイプ 2HA 活性を示す抗原は感染防御性を認めなかった。