Effect of Neutralizing Antibodies on Protection against Avian Reovirus Infection via the Footpad in Chickens Immunized with Killed or Live Virus-Antigen

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(Received 20 August 1991/Accepted 20 December 1991)

J. Vet. Med. Sci. 54(2): 383–386, 1992

KEY WORDS: avian reovirus, footpad lesion, neutralizing antibody.

Avian reoviruses (ARVs) are well known to cause tenosynovitis in young chickens [6, 8, 9]. Attempts to control tenosynovitis in chickens by vaccination have been based upon immunization of baby chicks since chickens are more susceptible to ARVs in the immediate post-hatching period and become increasingly resistant to infection with age [4, 5, 10, 17]. Therefore, passive immunity of progeny was conducted by parental vaccination with a live or killed virus-antigen of ARV in many works [2, 7, 13–15, 18]. As the results, the progeny from vaccinated flocks got maternal antibodies and resisted against oral challenge with virulent ARVs. But the progeny immunity was insufficient to protect against subcutaneous or footpad challenge with virulent ARVs. As to active immunity, there have been only a few reports describing that the young chicks vaccinated with attenuated live ARVs can be protected against subcutaneous [1] or footpad [3, 16] challenge. While, a role of neutralizing antibodies in protection has not yet been studied in their works.

On the other hand, the present authors reported that the footpad lesions, which were easily produced even in older chickens by the footpad route of infection, were useful parameters for the infectivity and the pathogenicity of ARVs [12].

In this work, we studied the effect of neutralizing antibodies on protection against footpad challenge in chickens immunized with killed or live-virus antigen of ARV.

Unsexed chickens of 14- and 30-day-old were derived from a specific pathogen free (SPF) chicken flock maintained at the authors' institute. Sera collected from the flock had no antibody to ARV. They were reared in isolated rooms and kept in stainless steel cages with wire floors during the experiments.

ARV, 58–132 strain [10], which was passaged 4 times in chicken kidney cells and 2 times in chicken embryonating eggs, was used for a live virus-antigen by oral treatment or a challenge virus by the footpad route. For a killed virus-antigen, the 58–132 strain was further passaged two times in chicken embryo fibroblasts, inactivated with 0.2% formalin and adsorbed on aluminium hydroxide gel as adjuvant [13].

Three experiments were conducted. In Expt. 1, forty 14-day-old chickens were divided into 4 groups. First (I-KKC) group was injected twice with 0.5 m*l* of the killed virus-antigen intramuscularly at 14- and 28-day-old. Second (I-LC) group received the live virus-antigen of $10^{4.0}$ PFU/bird of the 58–132 strain by the oral route at 14-day-old. The other two (I-CC and I-C) groups did not receive any virus-antigens. At 46-day-old, all chickens except for I-C group were challenged with $10^{4.0}$ PFU/bird of the 58–132 strain via the footpad route. Over a period of 14 days after challenge, the footpad lesions were observed by two methods as descrived previously [12] to record the lesion scores and the swelling indexes.

In Expt. 2, forty-eight 14-day-old chickens were divided into 5 groups. First (II-KC) group was injected with 0.5 m*l* of the killed virus-antigen intramuscularly at 14-day-old, while the second (II-KKC) group was injected twice at 14- and 28-day-old with the killed virus-antigen similarly as in II-KC group. Third (II-LC) group received the live virus-antigen of $10^{4.0}$ PFU/bird of the 58–132 strain orally at 21-day-old. Fourth (II-CC) and the last (II-C) groups did not receive any virus-antigens. At 46-day-old, all chickens were bled and challenged except for II-C group. Four days after challenge, footpad lesions were observed by two methods as in Expt. 1.

Sera were heated at 56°C for 30 min and tested for neutralizing antibodies against the 58–132 strain by the 90% plaque reduction methods [11].

In Expt. 3, eighteen chickens of 30-day-old received the live virus-antigen of $10^{4.0}$ PFU/bird of the 58–132 strain orally and three birds at 0, 2, 4, 6, 8, and 12 days after oral treatment were bled and challenged by the footpad route as in Expt. 1. Three untreated chickens were also challenged at 12 days. Their footpad lesions were judged 4 days after challenge by two methods as in Expt. 1. Sera were treated as in Expt. 2 to examine the neutralizing antibodies against the 58–132 strain.

Figure 1 shows the results of Expt. 1. I-CC group developed the severest footpad lesions showing the highest lesion scores and swelling indexes throughout the experimental period. On the other hand, I-KKC group developed milder lesions showing lower lesion scores and swelling indexes. Meanwhile, most chickens of I-LC group showed no gross lesions whose swelling indexes were less than 0.7 throughout the experimental period. Two or four days after challenge, only two birds showed very mild changes whose lesion scores were 1 and swelling indexes reached 0.8 and 1.2, respectively. Significant differences in the means of lesion score and swelling index were observed among three (I-KKC, I-LC and I-CC) groups challenged but not between I-LC and I-C groups, when compared at any time from 2 to 10 days after challenge.

Table 1 shows the results of Expt. 2. At 46-day-old, neutralizing antibodies were detected in all birds of three

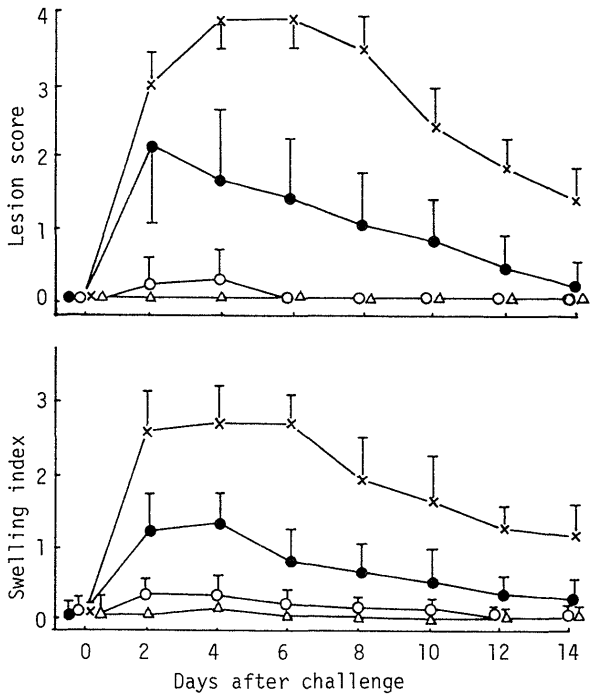

Fig. 1. Observation on footpad lesions after challenge by the footpad route with the 58–132 strain of avian reovirus (Expt. 1). Before challenge, I-KKC (●) group was injected twice with the killed virus-antigen and I-LC (○) group received the live virus-antigen. I-CC (×) group did not receive any virus-antigens before challenge. I-C (△) group was an unchallenged control.

(II-KC, II-KKC, and II-LC) groups which were treated with the killed or live virus-antigen. The geometric mean of neutralizing antibody titer was highest in II-KKC group (1:5,881), and those in II-KC and II-LC groups were same (1:788). Antibodies were not detected in II-CC and II-C groups which were not treated with any virus-antigens before challenge. The severity of gross footpad lesions after challenge decreased in the order of II-CC, II-KC, II-KKC and II-LC groups. Significant differences in the lesion scores and swelling indexes were observed among each group, but not between II-LC and II-C groups. These results coincided well with those of Expt. 1. Figure 2 shows the relationship between the neutralizing antibody titer at challenge and the footpad lesion score or swelling index of an individual bird of Expt. 2. Even the chickens having lower antibody titers showed a good protection. It was found that higher antibody titers were not always necessary to acquire a better protection.

Table 2 shows the result of an individual bird of Expt 3. A chicken (No. 6) out of three birds challenged at 2 days and every birds challenged at 4 days or later after oral treatment of the live virus-antigen showed low lesion scores and swelling indexes. Meanwhile, neutralizing antibodies began to appear 4 or 6 days after oral treatment, although they were at low level (1:80).

The authors' previous works showed that the killed virus-antigen could not provide a perfect protection against footpad challenge with virulent strains of ARV [11].

Meanwhile, Van der Heide *et al.* (1983) [16] and Haffer (1984) [3] have reported that one- or 6-day-old chicks vaccinated subcutaneously with attenuated live ARVs acquired a good protection against footpad challenge, although their neutralizing antibody levels at challenge were not determined.

In our present study, similar protective effects were observed. In Expts. 1 and 2, the killed virus-antigen induced insufficient protection against footpad challenge, nevertheless high levels of neutralizing antibodies were observed. In contrast, the live virus-antigen provided a good protection although it gave lower antibody titers than the killed virus-antigen.

As shown in Expt. 3, the protective effect induced by the live virus-antigen began to appear at the early time when the neutralizing antibodies were not yet detected. It is not clear whether this effect is specific or non-specific to

Table 1. Observation of footpad lesions after challenge by the footpad route with the 58–132 strain of avian reovirus (Expt. 2)

Group	Pre-challenge[a]		Neutralizing antibody titer at challenge[b]	Challenge	Post-challenge[c]	
	Virus-antigen	Age (days)			Lesion score	Swelling index
II-KC	Killed	14	788	+	2.30^{A}	1.52^{A}
II-KKC	Killed	14, 28	5,881	+	1.80^{A}	1.07^{B}
II-LC	Live	21	788	+	0.36^{B}	0.29^{C}
II-CC	—	—	<10	+	3.86^{C}	3.13^{D}
II-C	—	—	<10	−	0.00^{B}	0.24^{C}

a) The killed virus-antigen was injected intramuscularly and the live virus-antigen was inoculated orally at each age.
b) Geometric mean titers of chickens at 46-day-old.
c) Footpad lesions were observed 4 days after challenge and shown as means. Means in the same row with different superscripts are significantly different ($p<0.01$).

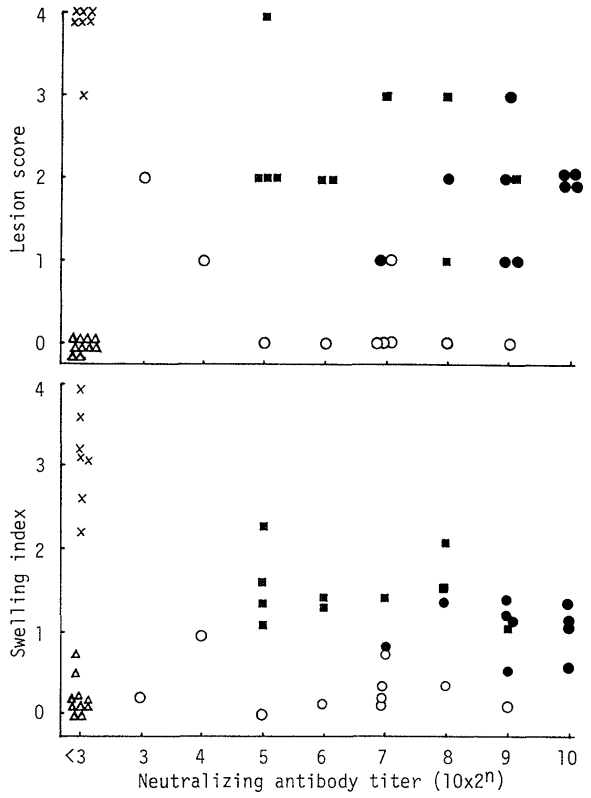

Fig. 2. Relationship between the neutralizing antibody titers at challenge and the footpad lesion scores or swelling indexes of chickens after challenge by the footpad route with the 58–132 strain of avian reovirus (Expt. 2). Groups II-KC (■), II-KKC (●) and II-LC (○) were immunized with the killed or live virus antigen, II-CC (×) was not treated with any virus-antigens before challenge, and II-C (△) was not challenged as shown in Table 1.

the live virus-antigen inoculated.

Thus, the neutralizing antibodies may have little correlation with resistance to footpad challenge, especially in chickens immunized with live virus-antigens. It is presumed that other immune mechanisms such as cellular immunity may mediate the protection in ARV infection. Further studies concerning this are necessary.

The 58–132 strain used in this study was very virulent and not attenuated to young chickens [10]. Therefore, it cannot be used for vaccine strain as it is, at once. For use as vaccine strain, it should be attenuated completely to avoid pathogenicity problems.

Table 2. An appearance time of protective effect against footpad challenge with the 58–132 strain of avian reovirus in chickens receiving the live virus-antigen (Expt. 3)

Days after treatment of live virus-antigen	Chicken no. tested	Neutralizing antibody titer at challenge	Footpad lesions 4 days after challenge	
			Lesion score	Swelling index
0	1	<10	4	3.85
	2	<10	4	3.53
	3	<10	4	2.46
2	4	<10	4	3.68
	5	<10	4	3.90
	6	<10	1	1.33
4	7	<10	2	0.89
	8	<10	2	1.39
	9	80	1	0.38
6	10	80	1	0.55
	11	80	1	0.75
	12	80	1	0.68
8	13	320	1	0.57
	14	320	0	0.20
	15	80	0	0.32
12	16	2,560	0	0.62
	17	640	1	0.92
	18	1,280	0	0.30
12[a)]	19	<10	4	3.31
(Un-	20	<10	4	4.00
treated)	21	<10	4	3.31

a) Three birds (Nos. 19, 20 and 21) were not treated with the live virus-antigen before challenge.

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