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Natural Anti-Ovalbumin Agglutinins in Adult Chickens[1] (39243)

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While testing for natural antibody in chicken sera, we noticed that some adult chicken sera reacted with ovalbumin (OA)[2] in a passive hemagglutinin assay. Since we also observed that very young chicks and specific pathogen-free (SPF) chickens lacked this activity, we surmised that the anti-ovalbumin (anti-OA) activity in the adult chicken sera may in fact be an antibody induced by environmental antigen, which incidentally cross-reacts with OA. Our purpose in these experiments was to examine this possibility more closely, particularly as this anti-OA agglutinin production might be related to age, environment, and breeding.

Materials and methods. Chickens. Inbred SPF (lines 7 and 100; ref. 1), conventionally reared inbred (Keystone line), and conventionally reared hybrid (Hy-Line-W-36) White Leghorn chickens were used. They were obtained from the United States Department of Agriculture Regional Poultry Research Laboratory, East Lansing, Michigan; Town Line Poultry Farm, Zeeland, Michigan, and Cook's Star Hatchery, Inc., Fremont, Michigan, respectively.

Aldehyde stabilized erythrocytes. White Leghorn chicken erythrocytes (CE) and pig erythrocytes (PE) were separated from fresh blood samples collected in Alsever's solution. The cells were washed five times in 0.11 *M* phosphate buffer (pH 7.2) and resuspended to 8% by volume. The procedure for stabilizing the erythrocytes with pyruvic aldehyde (P) followed by formaldehyde (F) treatment is described elsewhere (2). The stabilized erythrocytes, FPCE and FPPE, were stored at 4°.

Coating FPCE and FPPE with protein antigens. Crystalline bovine serum albumin (BSA) (Armour Pharmaceutical Co., Kankakee, Illinois) and crystallized ovalbumin (OA) (Worthington Biochemical Corp., Freehold, New Jersey) were used to coat FPCE. To 1 ml of a 1% suspension of FPCE in 0.1 *M* acetate buffer (pH 4), 400 μg of OA or BSA were added, and the mixture was stirred for 60 min at room temperature (2). The coated cells were washed five times in 10 vol of the 0.11 *M* phosphate buffer.

Sera. The chicken blood samples were allowed to clot 24 hr before serum collection.

Hemagglutinin assays. The chicken sera were diluted in a twofold series using the 0.11 *M* phosphate buffer containing 1 mg of gelatin (Difco Laboratories, Detroit, Michigan) per milliliter of buffer. In the conical wells of 96-place titration plates (Linbro Chemical Co., New Haven, Connecticut; Model IS-MVC-96), 25 μl of each of the serum dilutions was mixed with 25 μl of a 0.25% suspension of coated or uncoated stabilized erythrocytes. New pipets were used for each dilution to prevent "carry over." The trays were shaken twice at 5-min intervals and incubated at 4° until the patterns were read 18 to 20 hr later. Titer was taken as the highest dilution showing positive agglutination.

Specificity. The specificity of hemagglutination (HA) reaction was determined by absorption tests. To 50 μl of packed cells, OA, or BSA-coated FPCE or FPPE, 250 μl of the respective chicken serum was added,

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[2] Abbreviations used in this paper: BSA, bovine serum albumin; BSA-FPCE, BSA-coated FPCE; CE, chicken erythrocyte; F, formaldehyde; FPCE, chicken erythrocytes stabilized by pyruvic aldehyde followed by formaldehyde; FPPE, pig erythrocytes stabilized by pyruvic aldehyde followed by formaldehyde; OA, ovalbumin; P, pyruvic aldehyde; PE, pig erythrocyte; SPF, specific pathogen free.

TABLE I. THE PRESENCE OF NATURAL ANTI-OA AGGLUTININ IN SPF AND CONVENTIONAL CHICKENS.

Chicken group	Age	Sex	Breeding	Number of sera positive[a] titered vs OA-FPCE
SPF[b]	6 weeks[c]	ND[d]	Inbred	0/14
	19–22 months	3 ♂		0/3
		9 ♀		0/9
SPF Conventional[e]	13 weeks[c, f]	ND	Inbred	9/13
Conventional[e]	2 days	ND	Inbred	0/24
	10 weeks	2 ♂		2/2
		12 ♀		5/12
	19–22 months	4 ♂		4/4
	42 months	7 ♀		1/7
Conventional	22 weeks[c]	25 ♀	Hybrid	0/25
	33 weeks[c]	25 ♀		1/25
	17 months	8 ♀		0/8

[a] The reaction was considered positive when the titer vs the coated cells was at least three twofold dilutions higher than uncoated cells.

[b] Specific pathogen-free.

[c] SPF 6 weeks of age and SPF → Conventional 13 weeks are the same chickens. Conventional 22 and 33 weeks are the same chickens.

[d] Not determined.

[e] Chickens housed in a conventional environment.

[f] Six weeks in SPF isolation and 7 weeks in conventional environment.

incubated at 0° for 10 min, centrifuged, and the supernatant was tested with FPCE, OA-FPCE, BSA-FPCE, and FPPE at varying dilutions of the supernatant.

Results. The sera of inbred SPF chick, 6 weeks or 19–22 months old, did not contain any detectable anti-OA antibody (Table I). Similarly, sera of 2-day-old conventionally reared chick lacked the antibody. In contrast, conventionally reared adult inbred chickens had high anti-OA titers. In older chickens (42 months) the incidence of detectable anti-OA agglutinin was low.

The lack of anti-OA activity in the SPF chickens was not due to some inherent inability to produce the agglutinin since 6-week-old SPF chickens, which were transferred to a conventional housing, developed high titers of anti-OA within 7 weeks. In addition, all animals tested had agglutinins against pig erythrocytes (Table II).

Conventional rearing does not guarantee the development of anti-OA serum antibody, however, since conventionally reared adult chickens of a hybrid line also failed, with one exception, to produce detectable anti-OA agglutinin.

The presence of natural anti-OA agglutinin did not prevent egg-laying, but did delay the onset about 3 weeks in those raised under laboratory conditions.

The anti-OA agglutinin was specific as determined by absorbing sera with OA-FPCE, BSA-FPCE, or FPPE and titered against OA-FPCE, BSA-FPCE, or FPPE (Table II).

Discussion. The presence of natural anti-OA agglutinins in adult chickens may be the result of stimulation by cross-reacting environmental antigens because the young and SPF chickens did not have anti-OA activity. Since inbred SPF chickens, after being housed in a conventional manner for 7 weeks, did develop anti-OA agglutinins, pathogens[3] may be suspect as being the immunogens. The inbred line of SPF adult chickens kept in isolation remained negative for anti-OA activity.

The commerical OA used here most likely was derived from hybrid chickens and, if so, the nonreactivity of the hybrid to the commercial OA may not be surprising because hybrids could well have been rendered immunologically tolerant to the environmental antigens to which they were exposed since the perinatal period. Ideally the test OA should have been derived from White Leghorns, and we are currently in the process of conducting the experiment.

With the chickens reported in this paper, inbred lines with high anti-OA agglutinin

[3] By pathogens we mean the sum of antigens diminished due to the pathogen-free environment.

TABLE II. SPECIFICITY OF THE NATURAL ANTI-OA REACTION: SPECIFIC INHIBITION OF HEMAGGLUTINATION REACTION BY ABSORPTION OF SERA[a] WITH OA-FPCE, BSA-FPCE, OR FPPE TITERED AGAINST OA-FPCE, BSA-FPCE, OR FPPE.

Chicken group	Age	Sex	Absorbed with	HA titer vs OA-FPCE	Absorbed with	HA titer vs OA-FPCE	HA titer vs BSA-FPCE	Absorbed with	HA titer vs OA-FPCE	HA titer vs FPPE
SPF[b]	6 weeks[c]	ND[d]	None	8	None	8	8	None	8	256
			OA-FPCE	8	BSA-FPCE	8	8	FPPE	8	8
SPF	19–22 months	♀	None	8	None	8	8	None	8	64
			OA-FPCE	8	BSA-FPCE	8	8	FPPE	8	8
SPF → Conventional[e]	13 weeks[c, f]	ND	None	64	None	64	16	None	128	128
			OA-FPCE	8	BSA-FPCE	64	8	FPPE	128	8
Conventional[e]	10 weeks	♀	None	64	None	64	16	None	128	128
			OA-FPCE	8	BSA-FPCE	64	8	FPPE	128	8
	10 weeks	♂	None	64	None	64	16	None	128	128
			OA-FPCE	8	BSA-FPCE	64	8	FPPE	128	16
	19–22 months	♀	None	64	None	64	16	None	128	128
			OA-FPCE	8	BSA-FPCE	64	8	FPPE	128	16

[a] The serum of a single representative chicken from groups in Table I.
[b] Specific pathogen-free.
[c] SPF 6 weeks of age and SPF → Conv. 13 weeks are the same chickens. Conventional 22 and 33 weeks are the same chickens.
[d] Not determined.
[e] Chickens housed in a conventional manner.
[f] Six weeks in SPF isolator and 7 weeks in conventional environment.

titers reached sexual maturity 3 to 6 weeks later than the hybrid line of chickens, which did not show anti-OA agglutinin titers. Thus, the effect of anti-OA on egg-laying was suspected.

Because the chickens that manifested the high titers were from the same commercial breeder and the chickens which tested negative were from a different commercial breeder, genetic or environmental factors were also considered.

The specificity of the reaction was established by absorption experiments whereby serum was exposed to OA-coated FPCE and BSA-coated FPCE, comparing the HA titers before and after absorption. That these SPF chickens were immunocompetent is clear from the demonstration of specific agglutinin, which reacts with and can be absorbed by pig erythrocytes (Table II).

Summary. Sera from inbred SPF White Leghorn chickens at 6 weeks or 19–22 months of age did not have anti-OA agglutinins as determined by the passive HA reaction; 2-day-old chicks also lacked the anti-OA activity. Inbred chickens kept in SPF isolators for 6 weeks, then transferred to a conventional environment for 7 weeks, produced anti-OA agglutinins. Inbred White Leghorn adult chickens conventionally reared had a high anti-OA agglutinin titer. It appeared that natural anti-OA agglutinins are elaborated in response to environmental pathogens in certain lines of chickens. The titer in the individual chicken appeared as early as 10 weeks and was maintained until old age. The presence of natural anti-OA agglutinins did not prevent egg-laying but appears to have delayed it.

The HA reaction is specific and can be absorbed.

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