Effect of Blood Replacement on Passive Cutaneous Anaphylactic Reaction in Chickens[1] (37562)

ANNA C. ETTINGER AND ARTHUR A. HIRATA

University of Detroit, School of Dentistry, Detroit, Michigan 48207; and Department of Molecular Biology, Abbott Laboratories, North Chicago, Illinois 60064

Passive cutaneous anaphylactic (PCA) reactions are readily obtained in young chicks and specific pathogen free (SPF) chickens with allogenic antiserum, but seldom elicited in adult chickens over 4 mo of age (1–5). We postulated previously (3–5), that as chicks grow older, they produce homocytotropic antibodies of various specificities, which preempt skin receptor sites and thus interfere with the attachment of parenterally administered antibodies. To investigate this possibility, adult germfree chickens were exposed to a conventional environment to determine if at some point in time, the ability to respond to PCA reactions may be lost. Blood exchange studies were designed to substantiate the above hypothesis.

Materials and Methods. Six germfree white leghorn hens, hatched and raised in an isolator for 6 mo, were obtained from Sprague–Dawley Laboratories, Madison, WI. After isolation the chickens were kept in a conventional environment for intervals of time up to 17 mo.

For the blood exchange experiments, donor blood was withdrawn with a syringe, mixed with 10 units of sodium heparin for each 9 ml of blood. The blood was kept in a 38° water bath during the process of exchange. In order to obtain sufficiently large volumes, chick or SPF blood was pooled. Depending on the size of the recipient, a No. 30 or 27 gauge needle was inserted in a toe or ankle vein for injection. The recipient was injected with 10 units of sodium heparin/ml blood calculated as 10% of body weight (6). A distal phalanx was removed on the opposite foot for blood withdrawal. An attempt was made to keep the volumes of input and withdrawal constant. The blood replacement was continued until approximately 100% of the blood volume was injected. Chickens were tested for PCA reactivity about 24 hr after blood transfusion.

To test for the PCA reactions, chicken anti-bovine gamma globulin sera (anti-BGG) and chicken anti-rabbit gamma globulin sera (anti-RGG) were obtained from adult white leghorn chickens as described previously (4). Ten microliters of the chicken anti-BGG or anti-RGG serum were injected intracutaneously into the wing web. Two hours later 0.6 mg of either bovine gamma globulin (BGG) or rabbit gamma globulin (RGG) was given intracardially, dissolved in 0.02 ml saline and added to 0.04 ml of 0.4% solution of Evans blue dye for each milliliter of blood.

The germfree chickens were retested for PCA reactions at various intervals of time up to 17 mo. The PCA testing was performed on the donor adult chickens 24 hr after the blood exchange.

Results. Six 6 mo old germfree chickens gave positive PCA reactions on the day they were removed from isolation (Table I). Each specific chicken was retested for its ability to respond to the PCA reaction after months of living in a conventional environment. PCA reactivity was not permanent. The reactivity was lost as early as 1 mo or lasted as long as 12 mo in some chickens.

When a part of the blood of conventional adult chicken was replaced by blood of chicks

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TABLE I. PCA Reactions in Adult Germfree Chickens After Exposure to Conventional Environment.[a]

	Months held in conventional environment and wing web injections													
	0		1		4		6		10		12		17	
Chicken no.	Anti-BGG	Anti-RGG	Anti-BGG	Anti-RGG	Anti-BGG	Anti-RGG	Anti-BGG	Anti-RGG	Anti-BGG	Anti-RGG	Anti-BGG	Anti-RGG	Anti-BGG	Anti-RGG
7[b]	+													
8	+				+				—	+	+	+		
9	+			—			—		—	—				
10		+												
11		+	+					—	+		—	—	—	—
12		+			—				+		+	+	—	—

[a] For the PCA testing the homologous antigen or antigens was given intracardially. + = postive response; — = negative response to the homologous antigen.

[b] Germfree chickens were hens raised in the isolator until 6 mo of age.

or SPF chickens, the recipient gave a positive PCA reaction in 4 out of 4 and 2 out of 2 cases, respectively, when tested 24 hr after the blood exchange (Table II).

When a part of the blood of chick or SPF chicken was replaced by blood from a conventional adult chicken, 5 out of 6 and 3 out of 7 cases were PCA negative, respectively (Table II).

Discussion. The present work was undertaken to test the hypothesis that as chickens grow older in a conventional environment their skin receptor sites for homocytotropic antibodies are preempted by antibodies of various specificities. The finding that adult germfree chickens were PCA positive but lost their ability for PCA reactivity after living in a conventional environment supports the hypothesis. The hypothesis was further substantiated by the fact that blood from conventional adult chickens transfused into chick rendered the 7 day old chick PCA negative in 5 of the 6 cases tested where 50% negative response was expected at that age (4). Similar result was obtained when SPF chickens were used as blood recipients. When conventional adult chickens were used as blood recipients, unexpected results were obtained. A partial replacement of the blood of conventional adult chickens by blood of chicks or SPF chickens resulted in positive PCA reaction in all chickens tested. This 100% "conversion" is in contrast to the fact that only 3 out of 7 SPF chickens were converted by blood from conventional adult chickens.

The fact that blood from chicks and SPF can render the conventional adult PCA positive suggests that a PCA-facilitating factor exists in the blood. The fact that the

TABLE II. Blood Exchange Among Conventional Adult, SPF Chickens and Chicks.

Donor	Recipient	Expected percentage of PCA reactions of the recipient at comparable age (from previously reported data) (% neg)	PCA reactions in the recipent
Conventional adult	Chick[a]	50	5/6 neg
	SPF[b]	2	3/7 neg
Chick	Conventional adult	95	4/4 pos
SPF	Conventional adult	95	2/2 pos

[a] Chicks were 7 days old.

[b] SPF chickens were 6 mo old.

SPF chicken cannot be converted into a PCA negative condition as readily as the chick by conventional adult blood might be due to the ability of SPF chickens to produce the PCA-facilitating factor more rapidly than the chick. It appears then that PCA-inhibiting factors, including antibodies of various specificities mentioned above, and PCA-facilitating factors are both operative and that the relative abundance of one over the other influences the outcome of the PCA reactivity.

Summary. PCA reactions are seldom elicited in conventional adult chickens in contrast to the positive reaction that are readily obtained in young and specific pathogen free (SPF) chickens. Adult germfree chickens also gave postive PCA reactions. Blood exchange experiments were performed among conventional adult chickens, young and SPF chickens. Conventional adult chickens became PCA positive after receiving blood from young or SPF chickens. This suggests that a factor exists in the blood of young and SPF chickens that facilitates the PCA reaction.

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