A Chicken Sex-Limited Protein That Crossreacts with the Fourth Component of Complement

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ABSTRACT Plasma samples from more than 300 inbred chickens were screened by using an immunofixation technique with antibody against the fourth component of complement (C4) from humans. Precipitation patterns of plasma from adult male and sexually immature birds, either male or female, were identical. Plasma from egg-laying hens demonstrated a distinctly different precipitation pattern compared with plasma of other birds, with one additional band appearing 14 to 9 days before production of the first egg. The banding pattern could not be induced in males by progesterone injection and remained unchanged in molted female birds.

(*Key words:* sex-limited protein, immunofixation, components of complement)

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INTRODUCTION

The complement system includes a family of 20 or more serum proteins and cell surface receptors that participate in the effector function of the immune system (Reid and Porter, 1981). In mammals, the second (C2) and fourth (C4) components of complement, factor B (Bf), and a sex-limited protein (Slp) are encoded by genes of the major histocompatibility complex (Colten, 1984). The products of the Bf, C2, and C4 structural genes are characterized by codominant inheritance and extreme polymorphism (Grosse-Wilde *et al.*, 1983).

The sex-limited protein, a C4-like protein that lacks hemolytic acitvity, has been described in mice (Passmore and Shreffler, 1970) and hamsters (Coe, 1977). It was initially thought that the Slp was found exclusively in males of certain inbred strains of mice and that its presence in serum was under strict testosterone control. It has subsequently been described in female wild mice (Klein, 1975; Vergara, 1982) and female inbred mice (Brown and Shreffler, 1980), where the production of Slp depends on the presence of one or more permissive genes not linked to the major histocompatibility complex.

In a search for polymorphisms of the fourth component of complement (C4), we electrophoresed plasma from several different lines of inbred chickens into agarose gels and subsequently flooded those gels with goat antihuman C4 antibody. Each plasma tested exhibited two precipitin bands, but plasma from egg-laying hens demonstrated an additional or third precipitin band. This paper describes the identification of this precipitin band, physiological properties affecting it, and the lack of influence of progesterone on the induction of this protein.

MATERIALS AND METHODS

Birds and Management. Several inbred strains of chickens produced and maintained at the Poultry Science Research Center of Iowa State University were used in these experiments. Six to 12-week-old sexually immature chickens were exposed to a 10L:14D photoperiod. Five to 30-month-old hens and roosters were exposed to a 14L:10D photoperiod. Chickens were bled from the wing vein and plasma was isolated by centrifuging the blood at 1,000 × *g* for 10 min. Plasma samples were drawn at approximately 0800 hr. Each bird was sampled once, except as noted otherwise.

Immunofixation. Plasma samples were electrophoresed through a 1% agarose gel at 150 V until the plasma dye front had moved about 10 cm. The electrophoretic buffer used was Tris-

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glycine barbital buffer. Gels were flooded with goat antihuman C4 antibody (Atlantic Antibodies, Scarborough, ME). After incubation, gel was soaked for 1.5 hr in .9% NaCl to remove nonprecipitated proteins, stained in Coomassie blue for 1 hr, and detained overnight in methanol:acetic acid:dH_20 (50:10:40).

Hormone Assay. Estradiol levels were assayed by B. C. Wentworth (University of Wisconsin) using a radioimmunoassay procedure previously described (Wineland and Wentworth, 1975). Application of this assay to chickens has been validated (B. C. Wentworth, personal communication). Validation assay gave values of 121 ± 15 pg/ml for turkey (n = 4) and 140 ± 12 pg/ml for chicken (n = 6) plasma sampled 6 hr before ovulation. Crossreaction with 100 ng of added steroid was .3% for estriol and 1.5% for estrone. Interassay variation was 6.9%, and intrassay variation was 4.3%.

Induced Molting Procedure. Initially, food and water were withheld for 48 hr and then water was offered *ad libitum*, but food was withheld for an additional 8 days. No mortality or permanent adverse effects were noted as a result of this treatment in concurrence with findings that birds in the wild may fast for extended periods during incubation (Mrosovsky and Sherry, 1980).

In Vivo Progesterone Studies. Progesterone (10 mg/bird) in sesame oil was intramuscularly injected daily into 11 male birds for 7 days. An equal volume of sesame oil was given to 5 control birds. Birds were assayed on Days 0, 7, and 9 for the appearance of the third precipitin band by using the immunofixation procedure described.

Statistical Methods. A one-way analysis of variance was carried out using bird group as the classification. Means were separated by Duncan's multiple range test (Steel and Torrie, 1980). The correlation coefficient between age of appearance of the sex-limited precipitation band and age at first egg was calculated on an individual bird basis.

RESULTS

Plasma from all chickens examined gave at least two immunoprecipitation bands with the immunofixation procedure. Plasma from egg-laying hens, however, gave an additional band about 1 cm from the origin (Fig. 1). To determine the relationship of the additional band with age of onset of lay, serial plasma samples from 22 hens were examined twice weekly for appearance of the extra band beginning at 18 weeks of age. This band appeared 14 to 9 days before the production of the first egg (Fig. 2). The correlation between age at first egg and age at first band sighting was .92.

To determine whether the additional precipitation band was most closely associated with egg laying itself or with the hormones associated with laying, 14 2-year-old hens were induced to molt to assure that they were not laying eggs. Serial plasma samples were assayed by using the immunofixation procedure on Days 0, 2, 4, 6, 8, and 10 of the molt. The sex-limited banding pattern remained present in the plasmas of the 4 hens that had been laying eggs and had demonstrated the band before molting. One month later their plasma was again assayed for the sex-limited band. Eight of the 14 hens now showed the sex-limited precipitin band. These 8 hens were the only ones that produced eggs after the molt.

The plasma hormone levels of the hens on Day 10 of the molt and of sexually immature

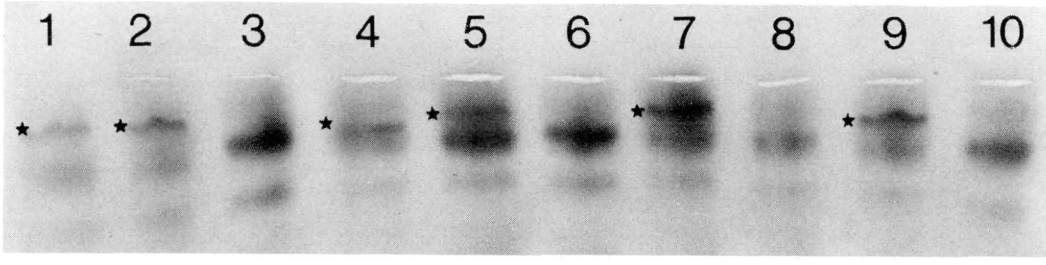

FIG. 1. Lanes 1, 2, 4, 5, 7, and 9 contain plasma from egg-laying hens. Only these lanes contain the sex-limited precipitate band (marked with asterisk). Lanes 3 and 6 contain plasma from adult male chickens. Lanes 8 and 10 contain plasma from sexually immature chickens. Plasma was electrophoresed at 150 V through a 1% agarose gel. Gel was flooded with goat antihuman C4 antiserum. After 1 hr at 37 C, gel was washed in .9% NaCl, stained with Coomassie blue, and then destained in methanol:dH20:acetic acid.

FIG. 2. Age in days at which the sex-linked precipitation band appeared was determined by use of the gel immunofixation technique described in Figure 1. This age is plotted against the age in days at which the chicken laid its first egg. The correlation coefficient for these two sets of data is .92.

chickens and egg-laying hens were also measured to identify a possible hormonal relationship between the sex-limited banding pattern and egg laying (Table 1). Estradiol levels in egg-laying hens (all of which exhibited the Slp band) and nonlaying molted hens that exhibited the Slp band were significantly different from estradiol levels of immature birds (none of which had the Slp band) and nonlaying molted hens not exhibiting the Slp band. Progesterone injection into roosters as described did not induce Slp expression.

DISCUSSION

After surveying more than 300 inbred chickens of various major histocompatibility types, we have been unable to demonstrate associated C4 polymorphisms by using immunofixation. However, a sex-limited protein was found in plasma of sexually mature females by using immunofixation with antihuman C4 antiserum. Unique proteins in laying hen serum that can be identified by precipitin tests and other immunological techniques have been previously noted (Sasaki, 1932; Roepke and Bushnell, 1936). Our studies, however, were the first to describe immunofixation of a unique protein band in laying-hen plasma with anti-C4 antibody. This suggested a structural and antigenic similarity of the Slp described in mice with that which we have described in chickens. Because females of the avian species are heterogametic, this suggested that a phylogenetic association of Slp with the heterogametic sex has persisted through evolution. The strong correlation between the onset of egg laying and appearance of the Slp precipitin band in the chicken was further evidence of the similarity between the hormone-dependent regulatory mechanism of Slp in the mouse and that in the chicken.

Earlier, David (1971) described a hormone-influenced serum protein in the chicken that could be induced by progesterone treatment. In the present experiment, a progesterone injection regimen similar to that of the David protocol was followed. Because our progesterone-injected birds revealed no Slp production in response to the injections, these two serum proteins may be distinct.

The hormone-associated nature of the chicken Slp was characterized further in the molting experiment (Table 1). Immature birds,

TABLE 1. *Estradiol in chickens examined for sex-limited protein (Slp)*

Group	Slp band in plasma	Estradiol[1]	
		(pg/ml)	(n)
Immature birds	–	32.7 ± 24.3[a]	10
Egg laying	+	152.9 ± 37.2[b]	15
Molted hens	–	35.4 ± 32.6[a]	10
Molted hens	+	94.8 ± 49.0[b]	4

[a,b]Means bearing different superscripts are significantly different ($P<.05$).

[1]Mean ± standard deviation.

before the onset of egg production, did not possess the Slp band and had low levels of estradiol. Likewise, molted hens that had not been laying immediately preceding the molt also failed to show the Slp band and had low levels of estrogen. In contrast, egg-laying hens (all of which exhibited the SLP band) had significantly higher levels of estradiol, similar to those of molted hens that had been laying eggs immediately before molting. Even though all the molted hens were out of production at the time that the hormone levels were assayed, the estradiol levels of those hens that had previously shown the Slp band were similar to egg laying hens, and the Slp band persisted in the plasma of these individuals. In addition, after the molting procedure, only birds that commenced egg laying demonstrated the precipitin band.

A strong correlation has been reported between the level of Slp and the major histocompatibility S region haplotype of inbred strains of mice (Ferriera *et al.*, 1982). Greater insight into the chicken Slp will be obtained when the chicken major histocompatibility complex has been characterized more fully.

Finally, we conclude that these experiments demonstrate a sex-linked serum protein in the chicken that may be structurally and antigenically similar to the fourth component of complement. The correlation of the appearance of this protein with onset of egg production suggests that further investigations are warranted to determine the physiological significance of this protein.

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