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Harderian gland dependency of immunoglobulin A production in the lacrimal fluid of chicken

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SUMMARY

Involvement of the Harderian gland (HG) in the production of lacrimal immunoglobulin (especially IgA) was investigated. The lacrimal concentration of each immunoglobulin class was not affected by surgical bursectomy but was reduced by cyclophosphamide (CY) and testosterone (TP) treatments. Surgical removal of the Harderian gland caused a remarkable reduction of both the lacrimal concentration of each immunoglobulin class and the specific antibody titre, and IgA was almost undetectable. The lacrimal concentration of each immunoglobulin class, as well as the specific antibody titre, was not affected by surgical removal of the Lacrimal gland (LG). The route of antigen administration produced no difference in the class of lacrimal immunoglobulin produced. The results indicate that the production of immunoglobulin in chicken tears may be dependent on the HG and that lacrimal immunoglobulin may be synthesized and secreted locally in the HG. Lymphocytes of the HG are of bursa of Fabricius origin and are seeded into the HG prior to hatching and its lymphocytes do not appear to be involved in systemic immunity.

INTRODUCTION

The chicken Harderian gland (HG) is an external secretor existing along the orbital septum. Since the existence of a large number of plasma cells in the interstitial tissue of HG has been reported by Bang & Bang (1968), HG has drawn much attention as one of the immuno-related organs and has stimulated studies on its immunological function.

Albini & Wick (1974) found that 70–90% of HG lymphocytes are of bursa of Fabricius origin and 10% of thymus origin. They also reported that HG contained a large number of cells (B cells) having surface immunoglobulin (Albini & Wick, 1973; Glick *et al.*, 1977).

Furthermore, Mueller, Sato & Glick (1971) detected plaque-forming cells in chicken HG lymphocytes by eye-drop application of sheep red blood cells (SRBC). However, by i.v. administration of SRBC, plaque-forming cells were detected in the spleen but not in the HG. From this finding, HG is considered to have no function in systemic immunity but is a peripheral lymphoid tissue that governs local immune responses.

As described above, it is easily understood that HG lymphocytes govern local humoral immunity in the eyes. However, little is known of the production of immunoglobulin classes by HG lymphocytes.

The purpose of this study was to investigate the HG-dependence of lacrimal immunoglobulin production with special reference to the production of IgA.

Abbreviations: CY, cyclophosphamide; HG, Harderian gland; Ig, immunoglobulin(s); LG, Lacrimal gland; NDV, Newcastle disease virus; SB, surgical bursectomy; SHG, surgical removal of Harderian gland; SHG_0, neonatal surgical removal of Harderian gland; SLG, surgical removal of lacrimal gland; SLG_0, neonatal surgical removal of lacrimal gland; SRBC, sheep red blood cell; TP, testosterone propionate.

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MATERIALS AND METHODS

Chickens and procedure of immunosuppression

White Leghorn chickens (bred in this laboratory) of non-inbred strain Hy-Line and inbred strain Anthony and eggs were used. Surgical removal of the HG and Lacrimal gland (LG) was performed by the procedure of Neumann (1976) and Survashe & Aitken (1977) on the day of hatching.

Surgical bursectomies (SB) were performed on the day of hatching. Chemical bursectomies were performed by daily i.p. injection of 2·5 mg of cyclophosphamide (CY; Endoxan, Sionogi & Company Ltd, Osaka) dissolved in saline (25 mg/ml), for 4 consecutive days starting on the day of hatching. Testosterone treatment (i.e. hormonal bursectomies) was performed by allantoic inoculation of testosterone propionate (TP; Sigma Chemical Company, St Louis, MO). Eggs were injected with 4 mg of TP in 0·1 ml of corn oil on the 12 and 14 embryonical days of incubation.

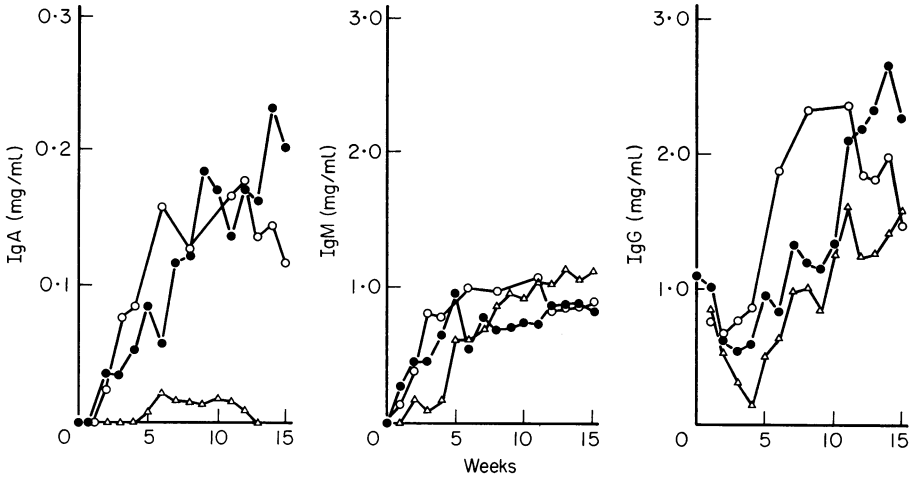

Figure 1. Immunoglobulin concentration in tears from normal and surgically or chemically bursectomized chickens. Concentration of lacrimal immunoglobulin classes from normal (●), SB (○) or CY (△) chickens were determined by SRID about 1–15 weeks of age.

Collection of lacrimal fluid (tear), saliva and serum

Stimulation of lacrimation in the chicks was performed by ocular instillation of 15 μl glycerin. Tears were collected from both eyes with pasteur capillary pipettes.

Stimulation of salivation was performed by the intramuscular injection of carbachol (0·7 mg/kg). Blood was withdrawn from the heart, and the serum heat inactivated at 56° for 30 min.

Preparation of immunoglobulins (Ig) and antisera

IgM was prepared from pooled chicken serum by precipitation with 25% saturated ammonium sulphate, gel filtration on Sephacryl S-300 and Sepharose CL-4B, DEAE–Sephacel (Pharmacia Fine Chemicals AB, Uppsala, Sweden) ion-exchange chromatography, and purified by adsorption to IgA coupled to CNBr-activated Sepharose immunoadsorbent. IgG Fc fragment was prepared from pooled sera by precipitation with 45% saturated ammonium sulphate, Sephacryl S-300 gel filtration, DEAE–Sephacel and CM–cellulose ion-exchange chromatography, treatment with papain, Sephacryl S-200 gel filtration and DEAE–Sephacel ion-exchange chromatography. IgA was prepared from pooled chicken bile by fractionation with 10% acetic acid, precipitation with 50% saturated ammonium sulphate, gel filtration on Sephacryl S-300 and Sepharose CL-4B and DEAE–Sephacel ion-exchange chromatography. The purity of each of the immunoglobulins was determined by immunodiffusion and immunoelectrophoresis using anti-chicken IgM (μ-chain specific), anti-chicken IgG and anti-chicken IgA (α-chain specific) (Miles Laboratories Inc., Elkhart, IN), and by polyacrylamide gel electrophoresis.

Antisera to each of the immunoglobulins were prepared in rabbits by subcutaneous injection of purified Ig incorporated into Freund's complete adjuvant. Booster injections of the same antigen were given a month later. The antisera were inactivated by heating at 56° for 30 min and adsorbed with IgG or IgG Fab′ coupled to a CNBr-activated Sepharose immunoadsorbent. These antisera were shown to be specific for the corresponding purified immunoglobulins by immunodiffusion and by immunoelectrophoresis.

Antigen, immunization and quantitative estimation of immunoglobulins and specific antibody titration

Freeze-dried live Hitchner B_1 vaccine strain of Newcastle disease virus (NDV; Nippon Biochemical Inst., Tokyo) was administered to both eyes of 3-week-old chickens with diminished passive antibody from maternal origin. The vaccine dose used was 10^5 50% embryo infective doses per bird. Tears, saliva and serum samples were collected at 1 and 2 weeks after immunization, i.e. at 4 and 5 weeks of age. The concentrations of Ig classes were determined by single radial immunodiffusion (SRID, realisable limit; IgA, 5 μg/ml; IgM and IgG, 25 μg/ml) and specific antibody to NDV (haemagglutination inhibition titre; HI titre) was determined by a microtitration.

Sheep red blood cells (SRBC) were washed three times, and 7% suspension of SRBC was administered by eye-drop (0·05 or 0·025 ml) three times at intervals of 3 days in 3-week-old chickens, and by intravenous injection (0·1 or 0·5 ml) of 4-week-old chickens. Tears, saliva and serum were collected at 5 weeks of age, the concentrations of immunoglobulin classes were measured by SRID, and specific antibody to SRBC (agglutinin antibody titre; HA titre) by microtitration.

RESULTS

Immunoglobulin classes in tears of non-immunized chickens

As shown in Fig. 1, IgA was detected from 2 weeks of age in the tears of normal chickens and levels increased with age and reached 0·2 mg/ml at 15 weeks of age. IgM appeared from 1 week of age and tended to increase until 5 weeks of age but later plateaued at approximately 0·8 mg/ml. IgG, which is initially of maternal origin and passively acquired, reached a minimum at 3 weeks of age. Subsequently, autogenous IgG gradually increased and reached approximately 2·3 mg/ml at 15 weeks of age. The pattern of secretion of these Ig classes was similar in both the Hy-Line and the Anthony strain.

The lacrimal IgA concentration in the SB chickens showed no significant variation with age. The lacrimal concentrations of

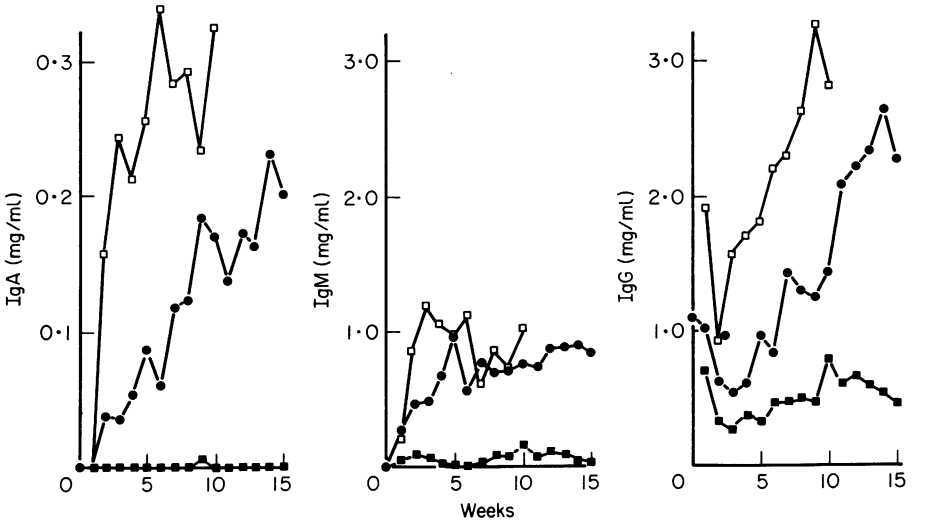

Figure 2. Immunoglobulin concentration in tears from chickens from which the HG or LG had been removed surgically. Concentration of lacrimal immunoglobulin classes from normal (●), SHG_0 (■) and SLG_0 (□) chickens were determined by SRID about 1–15 weeks of age.

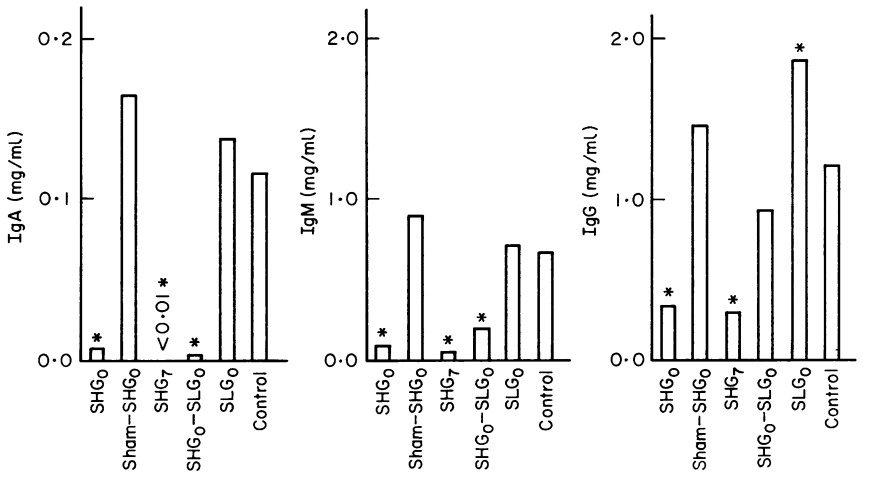

Figure 3. Immunoglobulin concentration in tears from chickens (from which the HG or LG had been removed) following ocular administration of NDV vaccine. NDV was administered at 3 weeks of age and the tears were collected at 1 and 2 weeks after administration (Control: normal; $*P < 0.05$).

IgM and IgG tended to increase in the SB chickens until 3–11 weeks of age compared with the normal chickens. After 13 weeks of age, however, it stayed either at the same or lower level as in the normal chickens.

IgA was almost undetectable in the tears of the CY-treated chickens. In these chickens, IgM stayed low until 5 weeks of age but later increased to the same or a rather higher level compared to the normal chickens. IgG levels were low at all ages. Though not shown in the figure, lacrimal Ig in chickens treated at 12 and 14 embryonic days with TP contained almost no IgA. In these chickens, both IgM and IgG showed almost the same pattern as in the CY-treated chickens.

Lacrimal concentrations of Ig classes in non-immunized chickens in which HG and LG were surgically removed at the neonatal stage

The significance of HG (which is considered to be a peripheral lymphoid tissue) and LG (which is a secretor of tears) in lacrimal secretion of Ig was then evaluated by surgical removal of these tissues (Fig. 2).

In the group treated by surgical removal of HG at the neonatal stage (SHG_0 group), all Ig classes were significantly reduced compared to the normal chickens and IgA was especially low. In contrast, in the group treated by surgical

Table 1. Immunoglobulin concentrations in saliva and serum from chickens (with surgically removed HG or LG) following eye-drop immunization of NDV vaccine

	Ig conc. (mg/ml)					
	Saliva			Serum		
Groups	IgA	IgM	IgG	IgA	IgM	IgG
SHG_0	<0·01	<0·01	0·26±0·11	0·02±0·02	0·89±0·22	3·16±0·48
Sham-SHG_0	0·01±0·01*	<0·01	0·28±0·07	<0·01	0·87±0·08	3·21±0·64
SHG_7	<0·01	<0·01	0·41±0·18	<0·01	0·87±0·07	3·16±0·24
SHG_0-SLG_0	<0·01	<0·01	0·42±0·14†	0·05±0·01†	0·91±0·05	3·42±0·36†
SLG_0	0·03±0·03	0·11±0·14	0·41±0·35	0·05±0·01†	0·81±0·18	2·92±0·39
Control	0·02±0·02	0·08±0·09	0·30±0·11	0·01±0·01	0·84±0·09	2·85±0·52

* Mean±SD.
† $P<0·05$.

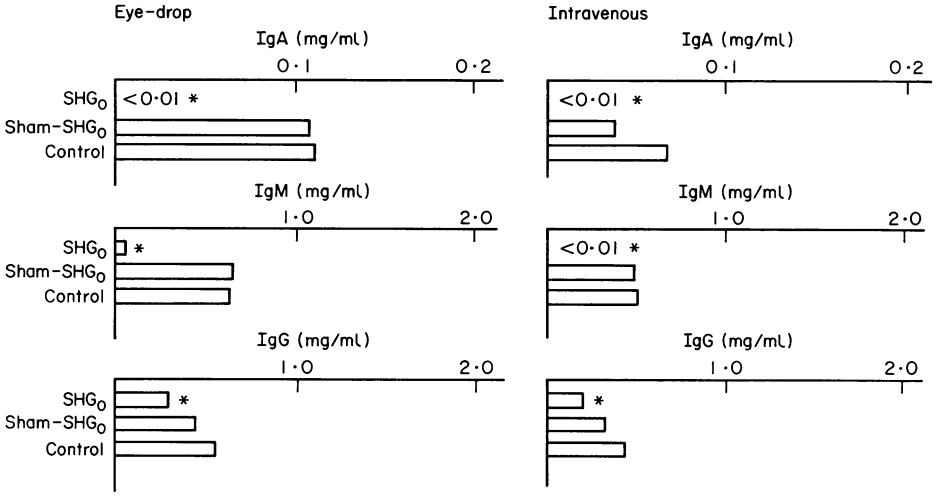

Figure 4. Comparison of immunoglobulin concentration in tears from chickens (from which the HG had been removed) following ocular and i.v. administration of SRBC. SRBC were administered by eye-drop three times at interval of 3 days in 3-week-old chickens, and by i.v. injection in 4-week-old chickens. The tears were collected at 5 weeks of age (Control: normal; $*P<0·05$).

removal of LG at the neonatal stage (SLG_0 group), the lacrimal concentrations of IgA, IgM and IgG increased significantly compared with normal chickens. However, the lacrimal fluid production was very low.

Production of lacrimal, salivary and serum Ig classes and specific antibodies in immunized chickens

Since identical results were obtained at 4 and 5 weeks of age in this experiment, only the results at 5 weeks are described. Six groups of chickens were studied: neonatal SHG (SHG_0) group, sham-SHG_0 group, 7-week-old SHG (SHG_7) group, neonatal SLG (SLG_0) group, neonatal SHG plus SLG (SHG_0-SLG_0) group and a non-treated (control) group.

The lacrimal concentrations of Ig classes are shown in Fig. 3. The concentration of each Ig class in the sham-SHG_0 group did not differ from that in the control group. However, the concentrations of IgA, IgM and IgG decreased significantly in the SHG_0 and SHG_7 group, IgA was almost entirely undetectable. In the SHG_0-SLG_0 group, IgA was almost undetectable and IgM was low, but IgG did not differ from that in the control group. In the SLG_0 group, IgA and IgM did not differ from those in the control group but IgG increased significantly compared to the control group.

The salivary concentrations of all Ig classes were much lower than the lacrimal concentrations. The SHG_0 and SHG_0-SLG_0 treatments did not contribute to further reduction of the concentrations (Table 1).

The serum concentration of IgA was lower compared to the lacrimal concentration. The SHG treatment induced no further reduction. Likewise, this treatment exerted no influence on the serum concentrations of IgM and IgG. In the SHG_0-SLG_0 group, the serum concentrations of IgA and IgG were higher compared to the control group. In the SLG_0 group, the serum IgA concentration was higher compared to the control group (Table 1).

The serum-specific antibody titres in the SHG_0-SLG_0 and SLG_0 group were higher compared to the control group.

However, no significant differences were found in the productions of Ig between the other immunized groups and the control group.

A similar experiment was carried out using SRBC because maternal antibodies to this antigen were considered to be non-existent. In addition, the effects of the route of antigen administration on the lacrimal antibody production were examined.

Regardless of the route of administration (eye-drop or i.v. injection), the SHG treatment resulted in low lacrimal concentrations of Ig classes, especially of IgA (Fig. 4).

DISCUSSION

The serum concentrations of Igs in normal chickens and also in surgically and hormonally bursectomized chickens is well documented. However, less attention has been given to the lacrimal concentration of Igs.

On examining the sequence of the appearance of lacrimal Ig classes, IgM was found to appear at first from approximately 1 week of age. This was followed with a slight delay by the appearance of IgA and IgG from 2 weeks of age. This phenomenon accords with the finding of Albini & Wick (1973) who studied the changes in the composition of Ig-bearing cells in HG.

On examining the behaviour of each lacrimal Ig class in the variously immunosuppressed conditions, neonatal SB exerted no influence on the production of IgA, IgM or IgG. CY and TP treatments had no influence on the production of IgM, but significantly reduced the production of IgG and resulted in almost no production of IgA. Assuming that this IgM was produced by lymphocytes of non-bursal origin, as reported by Lerner, Glick & McDuffie (1971), the results of TP treament indicate that antibody-producing precursor cells involved in the production of lacrimal Ig are cells of bursa of Fabricius origin, and the results of neonatal SB experiment indicate that all these cells had migrated into local areas of HG by the time of hatching.

The most remarkable inhibition of IgA production by the CY and TP treatments may suggest that the differentiation and maturation of IgA-producing precursor cells occurs later than those of IgM- and IgG-producing precursor cells. This conclusion is, however, contrary to the findings of Albini & Wick (1975) and Lebacq & Ritter (1979) who showed the presence of IgA-bearing cells in the bursa of Fabricius tissue of 10-day or 11-day embryos in which IgM- and IgG-bearing cells were still non-existent. This may indicate that the appearance of IgA-bearing cells constitutes a different mechanism which is not related to the differentiation and maturation of IgA-producing cells.

SHG at the neonatal stage induced almost no production of lacrimal IgA and IgM and caused significant reduction of IgG. This phenomenon also occurred even when SHG was performed at 17 weeks of age. In the tears of SHG chickens immunized with SRBC by eye-drop, a reduction of specific antibody titre, as well as a decrease in Ig, was clearly demonstrable.

These results strongly suggest the HG-dependence of lacrimal IgA. The relationship between the production of specific antibody and HG could not be adequately explained by the presence of residual passive antibody of maternal origin.

On examining the relationship between lacrimal Ig and LG, the concentration of each Ig class was increased by neonatal SLG in the non-immunized chickens. Following immunization with NDV by eye-drop, lacrimal IgG levels either remained normal or tended to increase. This seems to be the result of the concentration of Ig of HG origin as a result of SLG-induced reduction of lacrimal secretion.

The antibody-producing response of HG lymphocytes, which may be expressed by the relationship between the production of lacrimal-specific antibody and the route of antigen administration, is illustrated in Fig. 4. The routes of administration, eye-drop or intravenous injection, produced no obvious difference in the amount of each Ig class. The lacrimal concentrations of all Ig classes were reduced by intravenous injection and were similar to those seen in the non-immunized chickens (Fig. 1). It seems that lacrimal IgA does not result from migration of serum IgA but is produced locally in the HG.

This conclusion is in agreement with the concept of Sullivan & Allansmith (1984) who showed in rats that lacrimal IgA was not derived by transfer from the blood plasma. It is also consistent with the reports of Mueller *et al.* (1971) and Powell, Aitken & Survashe (1979) who found that HG lymphocytes could not be converted into antibody-producing cells by systemic (i.v.) administration of antigen.

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