Immunology, 1969, **17**, 325.

Immunoglobulin Synthesis and Lymphocyte Transformation by Anti-Immunoglobulin Sera in Bursectomized Chickens

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(*Received* 18*th September* 1968)

Summary. It was found in bursectomized chickens that selective inhibition of IgM or IgG immunoglobulin synthesis occurs with equal frequency. Alterations in the immunoelectrophoretic pattern of immunoglobulins were described. Lymphocyte transformation induced by rabbit anti-IgM serum was inhibited in chickens lacking either IgM or IgG. Spleen lymphocytes from bursectomized chickens with a normal pattern of immunoglobulins exhibited transformation induced by anti-globulin serum like controls, despite a decreased humoral antibody response.

INTRODUCTION

Removal of the bursa of Fabricius in newly hatched chickens is followed by marked inhibition of the humoral antibody response (Mueller, Wolfe and Meyer, 1960; Warner, 1967). Bursectomy did not suppress delayed hypersensitivity reactions (Janković, Išvaneski, Milošević and Popešković, 1963) or the rejection of skin homografts (Warner, Szenberg and Burnet, 1962), but rejection of an allogeneic cell suspension was impaired (Papermaster, Friedman and Good, 1962). Marked reduction in serum γ-globulin levels in some hormonally bursectomized chicks was reported by Carey and Warner (1964) and surgical bursectomy followed by irradiation resulted in total lack of immunoglobulins (Cooper, Peterson, South and Good, 1966). Ortega and Der (1964) and Cooper *et al.* (1966) often observed reduction or lack of IgG in the presence of normal or even elevated levels of IgM, but reduction of IgM synthesis also occured (Warner, 1967).

According to the original concept of functional dissociation of the chicken immune system (Warner *et al.*, 1962), the humoral antibody response and immunoglobulin synthesis is under bursal control. However, the finding of impaired antibody response in chickens with normal immunoglobulin levels raised the question of what is the significance of the 'non-antibody' γ-globulin. It seems that the factors affecting alteration of immunoglobulin synthesis and the choice of molecular type, are not clear. To get more information about this point, the incidence of various impaired immunoglobulin patterns was evaluated in a relatively large number of bursectomized chickens. Another approach was to obtain data about the potential for differentiation of the immunoglobulin forming cells. For this purpose, lymphocyte transformation induced by heterologous anti-immunoglobulin sera (Sell and Gell, 1965) was studied.

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MATERIAL AND METHODS

White Leghorn chickens were bursectomized surgically (Peterson, Burmester, Fredrickson, Purchase and Good, 1964) on the day of hatching. The next day chickens were irradiated with 650 r from a ^{60}Co source. The conditions of irradiation were: field size—114·5 cm^2; dose rate—13·8 r/min.

Sera were obtained from chickens at the age of 3–6 weeks. Micro-immunoelectrophoresis was performed using rabbit antisera, prepared by immunization with chicken immunoglobulins in Freund's adjuvant. Multispecific (against all immunoglobulin types) or specifically absorbed anti-IgG and anti-IgM sera were used (Skamene and Iványi, 1969b).

The antibody assay with ΦX bacteriophage was described elsewhere (Hájek, 1966).

The methods of lymphocyte transformation using chicken spleen cells and rabbit anti-immunoglobulin sera were described in detail previously (Iványi, Skamene and Kurisu, to be published). Spleen cells purified from aggregated cells by differential sedimentation for 60 minutes at 37° were washed twice with MEM-Eagle's medium. This medium with 100 U/ml Penicillin, enriched with 300 mg/l glutamine and 10 per cent (v/v) heat-inactivated rabbit (normal or anti-immunoglobulin) serum was used for cell culture. To each culture of 2 ml medium, 10^7 spleen cells (>90 per cent lymphocytes) were added. The cultures were set up usually in triplicate. The degree of stimulation was measured by the rate of incorporation of [^{3}H]uridine or [^{14}C]thymidine into TCA-precipitable cell components. The [^{3}H]uridine pulse (5 μCi/culture) was given for 2 hours on the 2nd day of cultivation, and [^{14}C]thymidine (0·5 μCi/culture) was present for 20 hours from the 2nd day overnight. Radioactivity was measured on a Mark-I model Nuclear Chicago liquid scintillation spectrometer.

RESULTS

IMMUNOELECTROPHORETIC PATTERN OF SERA

All chicken sera were scored by multispecific anti-globulin rabbit serum, which could detect precipitin lines of IgM, IgG and IgA in the sera of normal chickens (Fig. 1) as described previously (Iványi *et al.*, 1966). Moreover, a line around the start basin (denoted as β_2X), presumably not of immunoglobulin nature was seen. Additional examination of most of the sera was performed by monospecific anti-IgM (anti-μ) and anti-IgG sera (Figs. 2 and 3). The anti-IgG serum was not absorbed with L chains and, therefore, in sera lacking IgG the precipitation of IgM occurred as a result of cross-reactivity with anti-L chain antibodies (Fig. 3, No. 4). The pattern of immunoglobulins in sera of some bursectomized chickens (Fig. 1, Nos. 1, 2, 4 and 5; Fig. 2, Nos. 1 and 2) was similar to that which can be obtained with sera from normal chickens. Sera with hypo-IgG-globulinaemia revealed precipitin lines with limited heterogeneity and variable electrophoretic mobility (Fig. 1, serum Nos. 3 and 7; Fig. 2, serum Nos. 4–7). There was variability in the content of IgG with more anodic mobility. Another precipitin line with limited diffusion toward the well, probably involving a high molecular weight protein was revealed between the IgM line and the start-basin (Fig. 1, serum No. 4). This line was absent in some sera of bursectomized chickens (Fig. 1, serum No. 6).

The total incidence of the main types of immunoglobulin pattern in bursectomized and irradiated chickens is summarized in Table 1. Alteration of any type was observed in 34

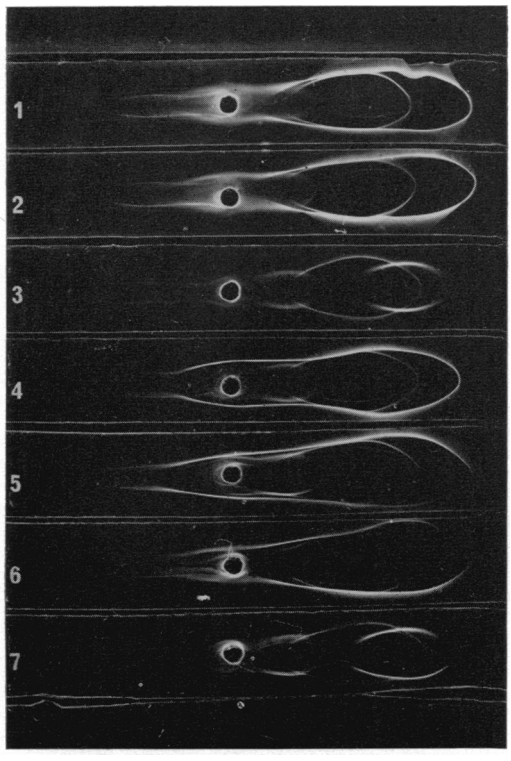

FIG. 1. Immunoelectrophoretic pattern of various sera from bursectomized chickens. Antiserum: Multispecific rabbit antiglobulin serum.

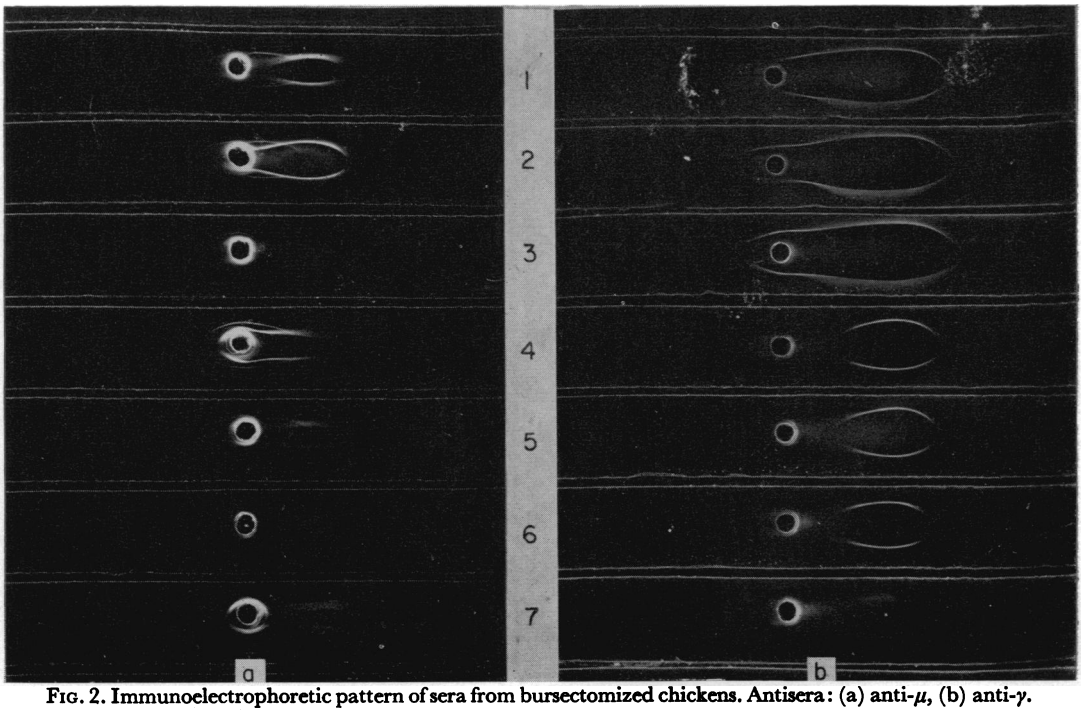

FIG. 2. Immunoelectrophoretic pattern of sera from bursectomized chickens. Antisera: (a) anti-μ, (b) anti-γ.

per cent of the sera. Absence or deficiency of IgG was observed in 17 per cent, absence or low IgM levels were present in 8·5 per cent and alteration of both molecular types (agamma- and hypo-γG) was found in 8·5 per cent of the chickens. Complete agammaglobulinaemia was not observed in any case.

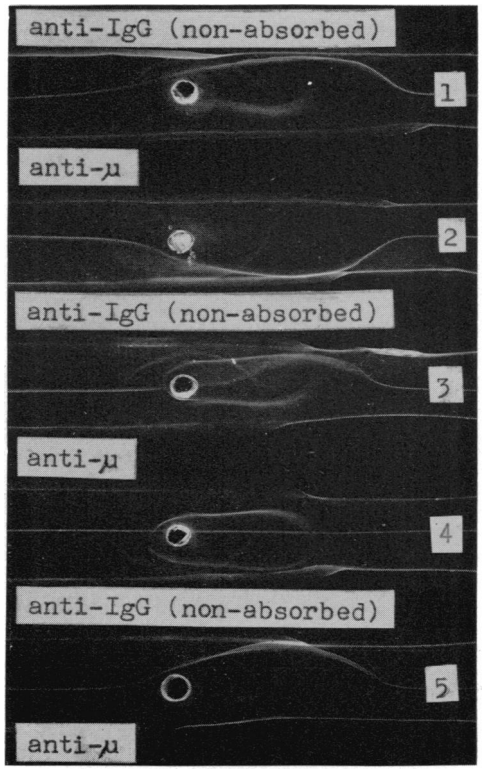

FIG. 3. Immunoelectrophoretic pattern of sera from bursectomized chickens: (1 and 3, normal; 2, agamma-M; 4, agamma-G; 5, hypo-γM). Antisera: anti-IgG (non-absorbed with L-chains); anti-μ.

TABLE 1

ALTERATIONS OF IMMUNOGLOBULIN SYNTHESIS IN BURSECTOMIZED-IRRADIATED CHICKENS

Normal pattern	Alteration of IgG		Alteration of IgM		Combined alteration	
	Absent	Decreased	Absent	Decreased	IgM absent	IgG decreased
101* (66%)	8	19	4	9		
	27 (17 per cent)		13 (8·5 per cent)		13 (8·5 per cent)	

Results of immunoelectrophoretic analysis of 154 chicken sera.
* No. of chickens.

STIMULATION OF RNA AND DNA SYNTHESIS IN SPLEEN LYMPHOCYTES

Spleen cells from bursectomized chickens lacking selectively either IgM or IgG were not stimulated in the presence of anti-IgM serum (Fig. 4). We failed to obtain stimulation in the presence of anti-γ chain serum in both cells from experimental and control birds. This finding is in accordance with other experiments (Skamene and Iványi, 1969a).

In another experiment the sera of bursectomized spleen cell donors had normal immunoelectrophoretic patterns. There was no significant difference between the response of these and control lymphocytes to multispecific anti-immunoglobulin serum (Fig. 5). However, earlier immunization of these chickens with 10^{10} ΦX174 bacteriophage particles showed a marked depression of anti-phage neutralizing antibodies.

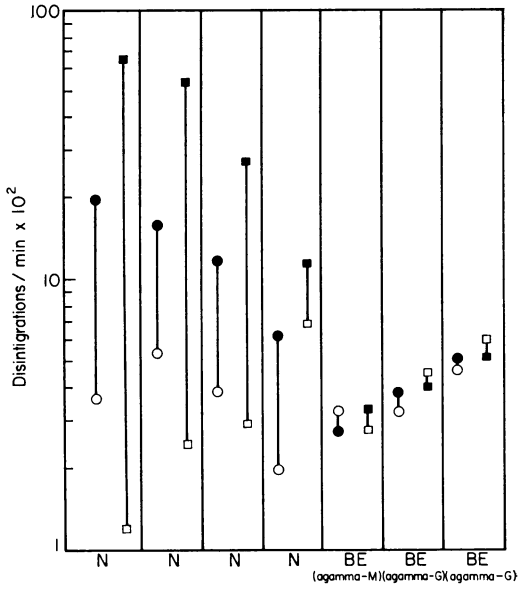

FIG. 4

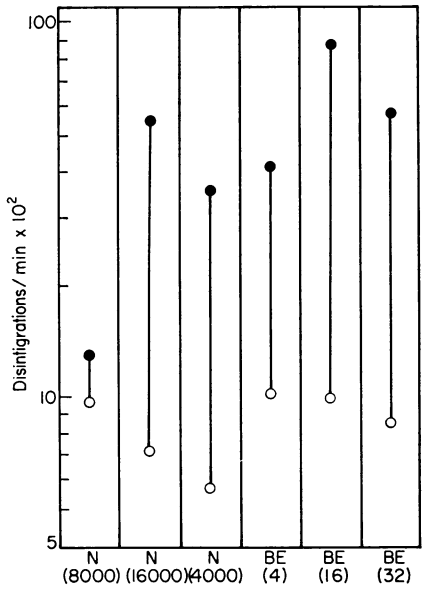

FIG. 5

FIG. 4. Rate of RNA and DNA synthesis in spleen lymphocyte cultures. N, Normal chickens; BE, bursectomized chickens. [^{3}H]Uridine incorporation in cultures with normal (○) or anti-IgM serum (●). [^{14}C]Thymidine incorporation in cultures with normal (□) or anti-IgM serum (■).

FIG. 5. Rate of DNA synthesis in spleen lymphocyte cultures. N, Normal chickens; BE, bursectomized chickens. [^{14}C]Thymidine incorporation in cultures with normal (○) or antiglobulin (●) serum. The data in parentheses indicate the serum titre of phage-neutralizing antibodies of the lymphocyte donors on the 7th day after injection with 10^{10} ΦX174 phage particles. Lymphocyte transformation was performed 2 weeks after immunization.

DISCUSSION

Immunoelectrophoretic examination of sera from bursectomized chickens has demonstrated a decrease or absence of IgM in a high number of dysgammaglobulinaemic chickens. Altered IgM synthesis was found in several birds with a normal level of IgG. Despite the limited quantitative significance of our data obtained by immunoelectrophoresis, there is no doubt about the active synthesis of IgG in 1–2-month-old chickens. It is improbable that IgG of maternal origin would persist sufficiently long to maintain the IgG at a normal level. This is also supported by the relatively rapid (2–3 days) half-life of chicken γ-globulin (Patterson, Younger, Wringle and Dixon, 1962; Iványi, Hraba and Černý, 1964) in comparison with some mammalian species. Our results are thus at variance with others, who found decreased IgG and normal IgM levels in bursectomized chickens (Ortega and Der, 1964; Cooper *et al.*, 1966; Van Alten, Cain, Good and Cooper, 1968).

Pierce, Chubb and Long (1966) noted that the residual IgG in some bursectomized chickens showed a restricted electrophoretic mobility. No difference was, however, found in either L-chain or H-chain banding patterns of IgG from bursectomized and normal chickens (Gold and Benedict, 1967). Also in humans with hypogammaglobulinaemic syndromes, qualitative abnormalities of IgG were found. Hong and Good (1967) demonstrated restricted and variable electrophoretic mobilities, differences in dye-binding capacity and Pickering, Hong and Good (1967) found deficient complement-fixing capacity of heat-aggregated IgG. Our results confirm the data on restricted electrophoretic heterogeneity of IgG and indicate a possible similarity in this respect between the chicken model and some human hypogammaglobulinaemias.

Lymphocyte transformation *in vitro* by cells from bursectomized chickens was studied only by Menwissen, Bach, Van Alten and Good (1967), who found that their response to PHA was normal. They observed unimpaired response to PHA also with lymphocytes from patients with agammaglobulinaemia and suppose therefore that this reaction, like delayed hypersensitivity and reaction against allogeneic cells is thymus dependent. In other studies on lymphocyte transformation by PHA or antigens in patients with hypogammaglobulinaemia either unaltered (Ling and Soothill 1964; Cooperband, Rosen, Kibrick and Janeway, 1966; Bradley and Oppenheim, 1967), or decreased (Elves, Roath and Israels, 1964; Cline and Fudenberg, 1965; Tormey, Kamin and Fudenberg, 1967) response was found.

It was found, that with spleen lymphocytes from bursectomized and irradiated chickens which had a depressed humoral antibody response to ΦX bacteriophage but a normal pattern of serum immunoglobulins, stimulation *in vitro* by anti-immunoglobulin (multispecific) serum was unimpaired. However, in bursectomized-irradiated chickens lacking either IgM or IgG in their serum, lymphocyte stimulation *in vitro* by anti-IgM serum was completely inhibited. It is of interest, that in those lacking IgG but with normal level of IgM, although cells synthesizing IgM were present, their ability to show stimulation *in vitro* by anti-IgM serum was altered. This finding might be due to:

(a) Alteration the ability of lymphocytes to differentiate in response to stimulation by antiserum in spite of the presence of cells synthesizing immunoglobulins with the corresponding specificity. In this case the impairment might be concerned with the accessibility of the specific receptor or with other metabolic insufficiency as a result of bursectomy and irradiation.

(b) Absence of cells capable of responding to stimuli of all kinds including PHA. At the present time we cannot decide between the two alternative explanations, because the small number of spleen cells from young chickens was not sufficient to perform cultures with anti-immunoglobulin sera and PHA simultaneously. However, stimulation of lymphocytes by PHA from bursectomized-irradiated chickens in additional experiments (unpublished results) produced variable results, but inhibition was certainly obtained with lymphocytes of some chickens.

It seems that the intensity of functional alteration of immunologically competent cells in bursectomized and irradiated chickens may be of various degrees: the primary humoral antibody response as the most sensitive sign is regularly inhibited; the potential for transformation *in vitro* is less affected and inhibition of immunoglobulin synthesis reflects to the most advanced alteration of the immune system.

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