Clin. exp. Immunol. (1974) **17**, 637–646.

LOCAL PRODUCTION OF IMMUNOGLOBULIN IN THE THYROID GLAND OF OBESE STRAIN (OS) CHICKENS

K. SCHAUENSTEIN AND G. WICK

Institute for General and Experimental Pathology, University of Vienna, Austria

(*Received* 4 *January* 1974)

SUMMARY

Thyroid glands of Obese strain (OS) chickens with spontaneous hereditary autoimmune thyroiditis were studied by direct immunofluorescence (DIF) with an anti-chicken immunoglobulin–FITC conjugate for local immunoglobulin (Ig) production in plasma cells and germinal centres. Many plasma cells and most of the germinal centres showed positive staining in DIF. The Ig property of this stained material was verified by DIF blocking and indirect immunofluorescence (IIF) tests with specific unlabelled anti-chicken Ig sera. In a chronological DIF study of thyroid glands from OS chickens aged 1–18 weeks, Ig-producing plasma cells could be already detected in the 1st week of life. DIF tests with TRITC-labelled chicken thyroglobulin revealed positive staining of plasma cells with preferential localization in close proximity to, or even between, follicular epithelial cells, suggesting the anti-thyroglobulin autoantibody nature of at least some of the locally produced Ig.

Positive results in DIF and IIF tests performed on infiltrated thyroid glands of OS chickens immunized with bovine serum albumin indicated the capacity of many infiltrating lymphoid cells within the thyroid to respond to exogenous antigens too.

INTRODUCTION

Chickens from the Obese strain (OS) of White Leghorns develop a spontaneous hereditary autoimmune thyroiditis (SAT) (Cole, Kite & Witebsky, 1968; Wick, Sundick & Albini, 1973). This disease is characterized by clinical symptoms of hypothyroidism, lymphoid infiltration of the thyroid glands and circulating thyroglobulin autoantibodies (TG-AAB) (Witebsky *et al.*, 1969; Kite *et al.*, 1969b). SAT in the OS seems to be an apt animal model for human Hashimoto thyroiditis (Kite *et al.*, 1969b; Wick & Graf, 1972).

In previous studies it has been well established that the development of SAT is dependent on the bursa-dependent part of the immune system (Wick *et al.*, 1970a). This is in contrast to findings in experimentally induced autoimmune diseases, such as experimental autoimmune thyroiditis and encephalomyelitis, the development of which is known to be dependent on an intact thymus-dependent portion of the immune system (Janković & Išvanevski, 1963; Janković *et al.*, 1965; Blaw, Cooper & Good, 1967).

Correspondence: Dr Georg Wick, Institute for General and Experimental Pathology, University of Vienna, A-1090 Vienna, Währinger Strasse 13, Austria.

The lymphoid infiltrations of the thyroid glands consist mainly of large pyroninophilic cells (mostly plasma cells) and numerous germinal centres (Kite *et al.*, 1969b; Wick *et al.*, 1970a). In the early stages of the infiltration process, plasma cells are found to abut the epithelium of degenerating or still intact thyroid follicles. The subsequent migration of plasma cells through the follicular basement membrane and between the epithelial cells—a process called periopolesis—is a very characteristic feature of SAT (Wick & Graf, 1972). On the basis of these and other data it has been postulated that B cells in the OS could have a dual function, one being immunoglobulin (Ig) production, the other a cellular type of immune reactivity (Wick *et al.*, 1970a; Wick & Graf, 1972).

There seems to exist a positive correlation between the presence of precipitating TG-AAB and the severity of SAT, but it still remains to be clarified if these AAB assume a primary pathogenetic role or if their occurrence is a secondary phenomenon.

In this respect it is noteworthy that TG-AAB could be shown to be vertically transmitted from the mother hen via egg yolk into the OS chicken embryo (Kite *et al.*, 1969a). A further resemblance of SAT to Hashimoto thyroiditis was that bound TG-AAB could be demonstrated in the thyroid *in vivo* glands of OS birds by means of immunofluorescence (IF) (Wick *et al.*, 1970b).

The present work was concerned with IF studies on thyroid glands of OS and normal White Leghorn (NWL) chickens in order to gain further insight into the immunopathological role of lymphoid cells engaged in thyroid infiltration in the former strain. It was attempted to answer the following questions using IF procedures. (1) Do plasma cells and germinal centres within the OS thyroid gland produce Ig? (2) If this is so, when can the first Ig-producing cells be detected? (3) Which Ig classes are produced? (4) Does local TG-AAB production occur in the thyroid gland? (5) Are plasma cells and germinal centres within the thyroid gland able to respond to external antigenic stimuli?

MATERIALS AND METHODS

Animals

IF tests were performed chronologically on thyroid sections of OS and NWL chickens aged 1–18 weeks. Both animal groups derived from our own flocks were hatched and raised in our own facilities as described elsewhere (Kite *et al.*, 1969b). Each chicken was sacrificed by heart puncture and the heat-inactivated sera were stored at −20°C until use. Both thyroid glands were harvested, one being fixed in buffered formaldehyde (pH 7·0) for routine histological analysis, the other snap-frozen and stored in liquid nitrogen. In some cases the spleen was also processed for IF.

Tissue sections and fixation

Cryostat sections (4 μm thick) of chicken thyroid glands and spleens, were air-dried at room temperature for 20 min and then subjected to various fixation procedures, such as methanol, ether, buffered formaldehyde or 5% acetic acid in 95% ethanol, at room temperature, 4°C or −20°C. The optimal method for the demonstration of Ig in thyroid glands proved to be the treatment of sections with a fixative consisting of equal volumes of 95% ethanol/ether for 15 min at room temperature. Local production and *in vivo* binding of TG-AAB was shown on sections of thyroid glands and spleen fixed in 10% buffered formaldehyde for 10 min. at room temperature.

IF reagents and procedures

Phosphate-buffered saline (PBS; pH 7·2) served as diluent and washing solution throughout.

(*a*) *Conjugates and unlabelled antisera.* Two anti-Ig conjugates were produced in our laboratory and characterized according to the guidelines of Beutner, Sepulveda & Barnett (1968) as described earlier (Wick *et al.*, 1970b). Conjugate C-W3 is the fluoresceinisothiocyanate (FITC) labelled γ-globulin fraction of a rabbit antiserum to chicken Ig (8 standard precipitation units of 1% protein/ml (U/ml); molar fluorescein/protein ratio (F/P) 2·2; dilution used 1:64).

Conjugate C-W1 is the FITC-labelled γ-globulin fraction of a goat antiserum to human IgG (8 U/ml; F/P 3·3; dilution used 1:64). Normal chicken TG prepared from saline thyroid extract, following a standard procedure (Witebsky *et al.*, 1969), was labelled with crystalline tetramethylrhodamineisothiocyanate (TRITC, Baltimore Biological Laboratories). An equal volume of TRITC dissolved in 2% $NaHCO_3$ (pH 8·2) was added dropwise (30 min at 4°C) to the TG solution at a fluorochrome to protein ratio of 1/100 (w/w). After stirring overnight at 4°C free dye was removed from the mixture by a passage over a Sephadex G-50 column (Pharmacia, Uppsala, Sweden) and the first protein peak harvested for further use. The optical density ratio (OD 280 nm/OD 550 nm) of the final conjugate was 12. Before application the antigenic properties of the conjugate were reassessed in passive haemagglutination and double diffusion tests using reference OS sera containing TG-AAB. For IF tests it usually was employed at a dilution of 1:4. Following another labelling procedure using acetone as diluent for the fluorochrome (Riggs *et al.*, 1958; Mellors, Broszko & Sonkin, 1962) a specific TG–TRITC conjugate could be prepared for which an OD ratio of 1·3 was calculated. In DIF this preparation was used at dilutions of 1:20 and 1:40.

C-WF9 is the FITC-labelled globulin fraction of a rabbit antiserum to bovine serum albumin (BSA) (this serum was kindly provided by Dr O. Förster). This conjugate had 16 precipitation U/ml, a F/P of 3·7 and was used at a dilution of 1:64.

F-458 K is a commercially obtained (Behringwerke, Marburg/L., Germany) FITC-labelled γ-globulin fraction of a goat antiserum to rabbit Ig (4 precipitation U/ml; F/P = 2·5; dilution used 1:64). Rabbit antisera specific for chicken μ- or γ-chains were prepared as described previously (Albini *et al.*, 1973).

An anti-normal chicken TG rabbit serum was produced by three weekly intracutaneous injections each consisting of 1 ml of an emulsion containing equal parts of 1% TG in PBS and Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan). The rabbit was bled 10 days after the last immunization.

(*b*) *IF tests.* (i) Local production of Ig was detected in direct IF (DIF) tests. Fixed tissue sections were washed immediately for 10 min at room temperature and the tests performed according to a standard procedure followed in this laboratory (Beutner *et al.*, 1968). For control purposes C-W3 was absorbed with lyophilized chicken Cohn fraction II (4 mg/ml) (Pentex, Kankakee, Illinois) for 60 min at room temperature and this material used in DIF tests.

(ii) The class of Ig produced by lymphoid cells within the infiltrated thyroid glands was determined in IIF tests treating fixed sections first with anti-chicken γ or μ rabbit antisera (diluted 1:16 to 1:64) and subsequently with the anti-rabbit Ig conjugate (F-458 K). Control experiments included the use of normal rabbit serum and an anti-human IgG conjugate (C-W1).

(iii) The TG-AAB nature of locally produced Ig was verified by IIF and DIF tests on sections of thyroid and spleen. For IIF the air-dried unfixed sections were pretreated with a 0·4% solution of normal chicken TG for 30 min at room temperature. After a 10-min rinse in PBS the sections were fixed in 10% formaldehyde for 10 min (to fix bound TG), washed in PBS for 10 min again and incubated with the anti-chicken TG rabbit-antiserum (diluted 1:32 to 1:256). Following a further 15-min wash the slides were treated with the anti-rabbit Ig conjugate. Controls included the use of PBS instead of TG, normal rabbit serum instead of anti-chicken TG and anti-human and anti-chicken IgG conjugates instead of the anti-rabbit Ig preparation. In DIF tests the fixed thyroid and spleen sections were covered with the TRITC-TG conjugate and the readings compared with those of the IIF tests and their controls.

(iv) The demonstration of local production of antibodies to exogenous antigens in infiltrated thyroid glands was carried out with OS chickens immunized by one intravenous injection of BSA (40 mg/kg) at an age of 12 weeks. Four weeks after the immunization the thyroid glands of those birds and of unimmunized age-matched OS control chickens were harvested and studied for the presence of BSA–antigen and anti-BSA antibodies. Fixed thyroid and spleen sections were treated with dilutions of BSA (0·5, 1·0, and 2·0 mg/ml) for 15 min and subsequently flooded with the anti-BSA conjugate for the demonstration of BSA antibodies. These slides were compared to sections treated with the anti-BSA conjugate only in DIF tests. For control purposes PBS was used instead of BSA and the anti-human and anti-chicken Ig conjugates instead of the anti-BSA conjugate.

Optical equipment

Readings were made on a Reichert-Fluoropan microscope equipped with a HBO-50 high pressure mercury vapour bulb, a FITC-3 exciter filter, a Schott KV 418 barrier filter and a dark ground condensor. For observations in incident light a Reichert-Zetopan microscope equipped as described earlier (Albini & Wick, 1973) was employed.

Histology

One of the two thyroid lobes of each chicken was processed for routine histology as described previously and the degree of SAT determined according to a standard scoring schedule ranging from 1+ (up to 25% of thyroid cross-section infiltrated) to 4+ (75%—total infiltration) (Kite *et al.*, 1969b).

RESULTS

Detection of Ig-producing plasma cells and germinal centres within OS-chickens thyroid glands

DIF tests on sections of OS thyroid glands using an anti-chicken Ig conjugate show a very characteristic picture, namely brillant staining of both many plasma cells and the germinal centres (Fig. 1). Positive staining was most commonly encountered in plasma cells either abutting thyroid follicles or migrating between follicular epithelial cells (periopolesis) (Fig. 2).

The remaining colloid within the follicles of the infiltrated thyroid glands often revealed *in vivo* bound Ig, most probably TG-AAB as demonstrated in previous investigations (Wick *et al.*, 1970b). The Ig nature of the stained material could be verified by specific blocking tests with unlabelled anti-chicken Ig sera and negative results after absorption of conjugate C-W3 with chicken Ig.

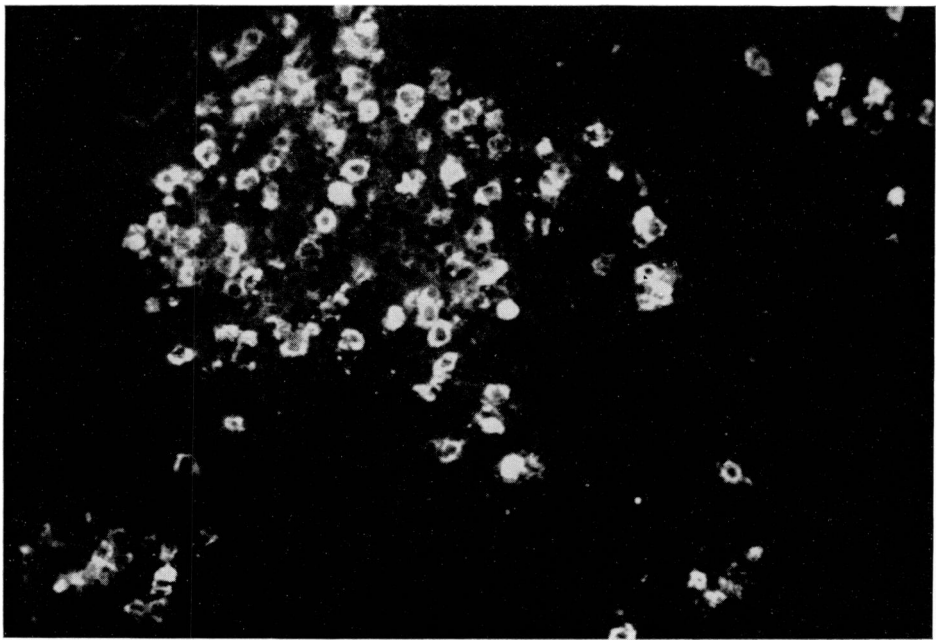

FIG. 1. Thyroid gland of a 10-week-old OS chicken. Direct immunofluorescent staining with the anti-chicken Ig FITC-conjugate reveals a distinct germinal centre and many plasma cells within totally infiltrated gland. (Magnification × 160.)

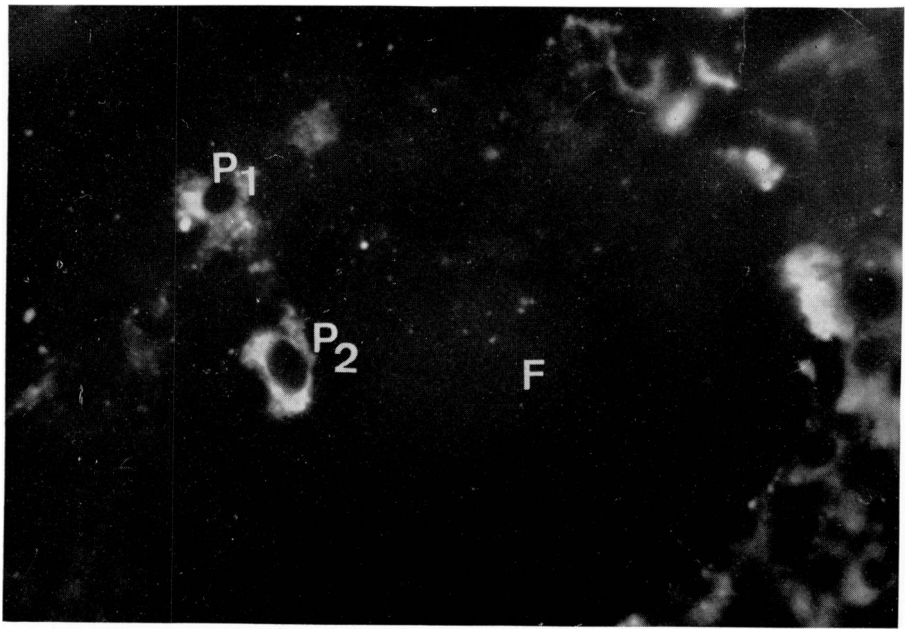

FIG. 2. Thyroid gland of a 4-week-old OS chicken. Direct immunofluorescent staining with the anti-chicken Ig FITC-conjugate shows one plasma cell (P_1) in process of periopolesis and another (P_2) already merged into follicular lumen (F). (Magnification × 630.)

Chronological studies

In order to study the kinetics of the local Ig production during the course of the disease, thyroid glands of OS and NWL chickens aged 1–18 weeks were tested chronologically in DIF tests. Table 1 lists the animal groups employed, their age, mean degree of SAT and presence of circulating TG-AAB. The increase of the severity of SAT as well as the frequency and titres of TG-AAB in OS chickens are obvious.

Ig-producing plasma cells could first be detected in the stroma of thyroid glands of 1-week-old OS-chickens. The first positively stained germinal centres were encountered at the age of 2 weeks.

TABLE 1. Degree of autoimmune thyroiditis and occurrence of circulating thyroglobulin autoantibodies in the group of chickens investigated

Age (weeks)	Strain	*n*	Thyroid histology	TG-autoantibodies	
				TCT ($10g_2$)	PPT
1	NWL	2	—	0	0/2
	OS	4	±	0·75	0/4
2	NWL	2	—	0	0/2
	OS	4	1·5	1·0	0/4
3	NWL	2	—	0	0/2
	OS	4	3·0	2·0	2/4
4	NWL	2	—	0	0/2
	OS	4	2·2	2·2	3/4
5	NWL	1	—	0	0/2
	OS	2	2·0	1·0	1/2
6–7	NWL	2	—	0	0/2
	OS	3	4·0	5·6	3/3
10–18	NWL	—	—	—	—
	OS	6	3·6	5·0	2/6

Scoring schedule for severity of thyroiditis: 0, no lymphoid infiltration; 1+, up to 25% of total thyroid cross-section infiltrated; 2+, 25–50%; 3+, 50–75%; 4+, 75% total infiltration.

Abbreviations: NWL, normal White Leghorn; OS, Obese strain; SAT, spontaneous autoimmune thyroditis; TG, thyroglobulin; TCT, tanned cell haemagglutination tests (mean titres); PPT, double diffusion in gel precipitation tests (number of positive sera/total number of sera).

Determination of the class of the locally produced Ig

IIF tests using antisera against chicken γ- and μ-chains performed on infiltrated thyroid glands and spleens of OS chickens revealed both IgG- and IgM-producing plasma cells and germinal centres in the thyroid as well as in the spleen, IgG being predominant.

Local TG-AAB production within the thyroid

In DIF tests positive staining with TRITC-labelled TG could be demonstrated in relatively few plasma cells only, with preferential location near thyroid follicles. Fig. 3 shows a plasma cell in the process of periopolesis which binds the labelled TG and may thus be engaged in TG-AAB production. Germinal centres were only rarely found to stain with the labelled TG. Both positive plasma cells and germinal centres also occurred in the spleen at the same low frequency. These findings were confirmed by DIF tests using a TG–TRITC

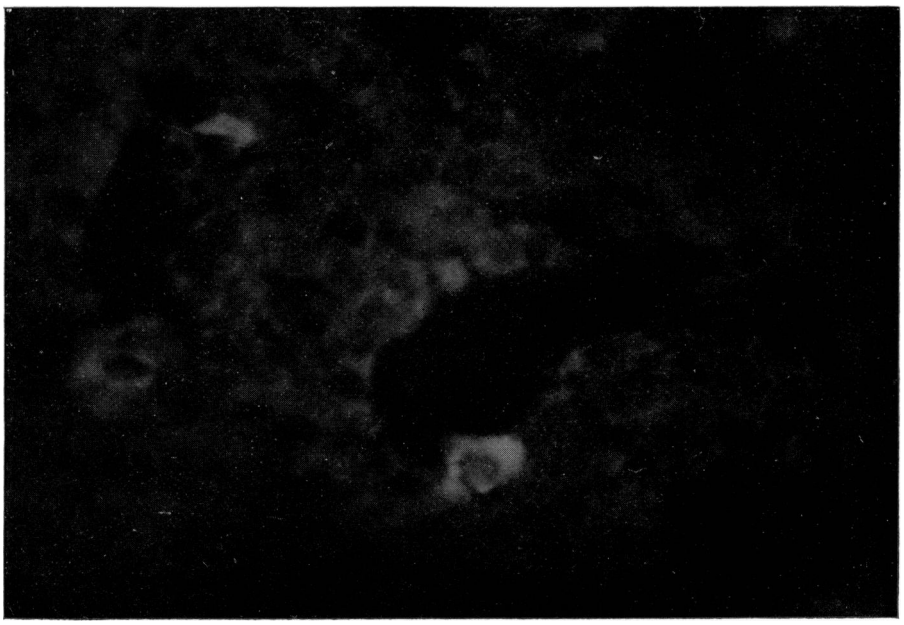

FIG. 3. Thyroid gland of a 4-week-old OS chicken. Direct immunofluorescent staining with a TRITC–chicken thyroglobulin conjugate. Note peripolesis of the thyroglobulin autoantibody-producing plasma cell. (Magnification × 630.)

conjugate with about a ten times higher degree of labelling. In addition, TG-AAB production could be shown using an IIF system where the sections were first treated with unlabelled TG, then with a rabbit antiserum to chicken TG and finally with an anti-rabbit Ig conjugate. Due to the increased sensitivity of IIF more plasma cells and germinal centres producing TG-AAB were found as compared to DIF tests and the colloid also showed brilliant fluorescence in these formaldehyde-fixed sections (Fig. 4).

Demonstration of local immunological response to exogenous antigens

IIF tests on infiltrated thyroids of OS chickens immunized with BSA showed high numbers of positively stained plasma cells and germinal centres (Fig. 5). The BSA, which was already present within germinal centres before the treatment of the sections with this antigen, was distinguished from the latter by means of parallel preparations treated with the anti-BSA conjugate in a DIF test only. While plasma cells, lymphoid cells of germinal centres and dendritic cells (with ingested BSA or BSA–anti-BSA complexes) showed positive staining

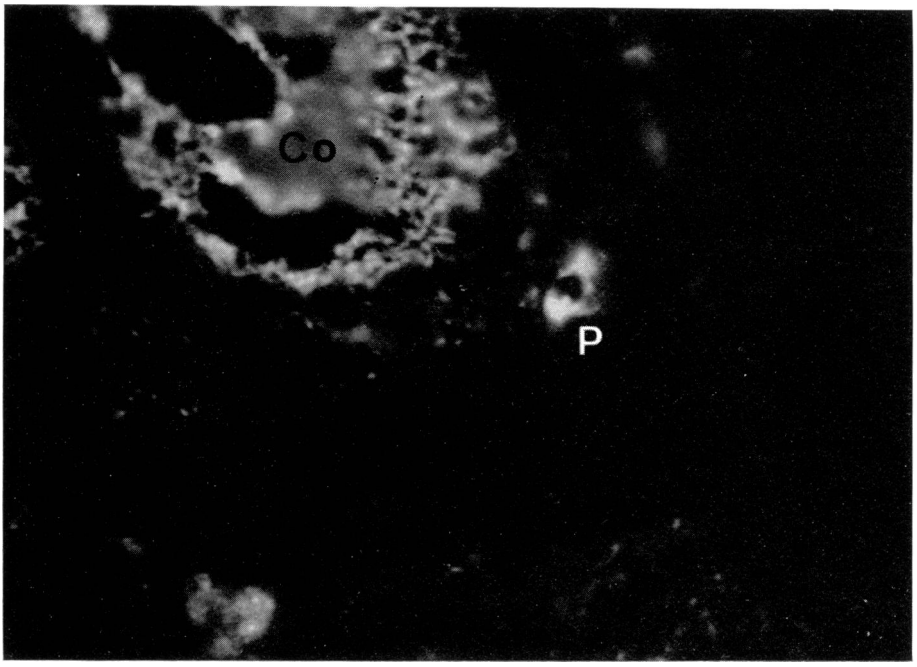

FIG. 4. Thyroid gland of a 10-week-old OS chicken. Indirect immunofluorescence test using unlabelled chicken thyroglobulin followed by a rabbit antiserum to chicken thyroglobulin and finally an anti-rabbit Ig–FITC conjugate. Note a thyroglobulin-binding plasma cell (P) and brilliant staining of pre-existing thyroglobulin in colloid (Co). (Magnification ×630.)

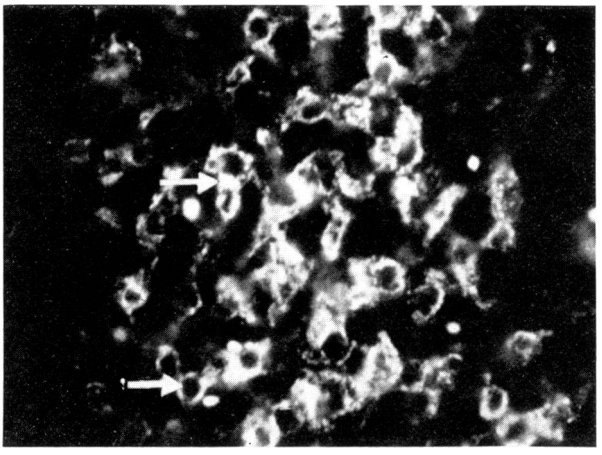

FIG. 5. Thyroid gland of a 16-week-old OS chicken which had been immunized with BSA 4 weeks before sacrifice. Indirect immunofluorescence test using unlabelled BSA and subsequently a rabbit anti-BSA–FITC conjugate. Fluorescence of both lymphoid cells producing antibodies to BSA (upper arrow) and dendritic cells (lower arrow) with *in vivo* bound antigen. (Magnification ×630.)

in IIF tests, only the latter were distinguished in DIF. These findings were also characteristic for those tests where spleen sections were employed.

DISCUSSION

Local Ig production within the thyroid gland of OS chickens has been proposed already in earlier studies (Wick *et al.*, 1970b) due to the occurrence of characteristically high numbers of plasma cells and germinal centres (Kite *et al.*, 1969b; Wick *et al.*, 1970a). The present study conclusively clarified this point and also supported earlier electron microscopic data which were suggestive of Ig production by plasma cells which are in close contact with the follicular epithelium or even migrate between epithelial cells (periopolesis). The role of TG-AAB in the development of SAT is not yet clear. It has been shown earlier that there seems to exist a correlation between the presence of precipitating TG-AAB and the severity of disease (Kite *et al.*, 1969b), but attempts to passively transfer SAT to NWL chickens by means of TG-antibody-containing OS sera were—so far—unsuccessful. The possible local requirement of such antibodies at the site of a B cell-mediated cellular type of immune reaction (perhaps antibody-mediated B-cell lymphocytotoxicity) remains, however, to be clarified. The assumption of a dual function of B cells in OS chickens, namely antibody formation, and a cellular type of immune reactivity, is supported by several previous findings (Wick *et al.*, 1970a; Wick & Graf, 1972; Wick & Steiner, 1972).

In view of the fact that SAT in the OS is a 'B-dependent' disease which can be suppressed by neonatal or *in ovo* bursectomy (Wick *et al.*, 1970a) and that plasma cells (i.e. cells from the B-cell line) are the first lymphoid cells to arrive in the thyroid stroma at 1 week of age, the chronological study of local Ig production was of special interest. Ig production of infiltrating plasma cells could be demonstrated already at 1 week of age, while the first Ig producing germinal centres appeared 1 week later and increased in frequency with increasing severity of thyroiditis.

The investigations concerning the class of locally produced Ig were not done in a chronological fashion, but performed on 9-week-old OS chickens with fully developed disease only. It will be of further interest to evaluate quantitatively the occurrence of IgG-, IgM- and IgA-producing cells respectively in the OS thyroid at different ages of life.

The proof of the TG-AAB nature of at least some of the locally produced Ig further emphasizes the similarity of the OS chicken model and human Hashimoto's disease (Mellors *et al.*, 1962). However, the relatively low number of TG-AAB-producing cells, albeit generally in close contact with the thyroid follicles, was somewhat surprising. At first this low incidence of TG-AAB producers was thought to be due to the low efficiency of TRITC-labelling of chicken TG which is obvious from the high OD ratio of 12 calculated for this conjugate. Attempts to establish more efficient labelling conditions for chicken TG were successful in recent experiments and led to a TG-TRITC conjugate with an OD ratio of 1·3. Neither DIF using this latter conjugate nor IIF methods, however, afforded considerably higher numbers of TG-binding plasma cells. The fact still remained that most of the cells did not produce TG-AAB. This observation should promote further studies for other autoantigens besides TG, possibly involved in the development of SAT. Up to now no such other antigens were identified.

Both antigen localization in dendritic cells of germinal centres and antibody production by lymphoid cells have been demonstrated in the thyroid glands of BSA-immunized OS

chickens. The strong local immune response to BSA supports the view that the severe lymphoid infiltration has transformed the thyroid gland into a peripheral lymphoid organ capable of responding to exogenous as well as to autoantigenic stimuli.

ACKNOWLEDGMENTS

This work was supported by the Austrian Research Council (project 1997). The authors wish to thank Dr W. Hijmans, Rijswijk—Netherlands, for critical discussions. The competent and reliable technical help of Miss R. Steiner is appreciated.

REFERENCES

ALBINI, B. & WICK, G. (1973) Immunoglobulin determinants on the surface of chicken lymphoid cells. *Int. Arch. Allergy*, **44**, 804.

ALBINI, B., WICK, G., ROSE, E. & ORLANS, E. (1974) Immunoglobulin production in chicken Harderian glands. *Int. Arch. Allergy* (In press.)

BEUTNER, E.H., SEPULVEDA, M.R. & BARNETT, E.V. (1968) Quantitative studies of immunofluorescent staining. Relationships of characteristics of unabsorbed antihuman IgG conjugates to their specific and nonspecific staining properties in an indirect test for antinuclear factors. *Bull. Wld Hlth Org.* **39**, 587.

BLAW, W.E., COOPER, M.D. & GOOD, R.A. (1967) Experimental allergic encephalomyelitis in agammaglobulinemic chickens. *Science (N.Y.)*, **158**, 1198.

COLE, R.K., KITE, J.H. & WITEBSKY, E. (1968) Hereditary autoimmune thyroiditis in the fowl. *Science*, **160**, 1357.

JANKOVIĆ, B. & IŠVANEVSKI, M. (1963) Experimental allergic encephalomyelitis in thymectomized, bursectomized and normal chickens. *Int. Arch. Allergy*, **23**, 188.

JANKOVIĆ, B.D., IŠVANEVSKI, M., POPESKOVIĆ, L. & MITROVIĆ K. (1965) Experimental allergic thyroiditis (and parathyroiditis) in neonatally thymectomized and bursectomized chickens. Participation of the thymus in the development of the disease. *Int. Arch. Allergy*, **26**, 18.

KITE, J.H., FLANAGAN, T.D., SUNDICK, R.S. & WITEBSKY, E. (1969a) Factors associated with the development of spontaneous autoimmune thyroiditis. *Fed. Proc.* **28**, 631.

KITE, J.H., WICK, G., TWAROG, B. & WITEBSKY, E. (1969b) Spontaneous thyroiditis in the Obese Strain of chickens. II. Investigations on the development of the disease. *J. Immunol.* **103**, 1331.

MELLORS, R.C., BRZOSKO, W.S. & SONKIN, L.J. (1962) Immunopathology of chronic nonspecific thyroiditis (autoimmune thyroiditis). *Amer. J. Path.* **41**, 425.

RIGGS, J.H., SEIWALD, R.J., BRUCKHALTER, J.H., DOWNS, C.M. & METCALF, J.G. (1958) Isothiocyanate compounds as fluorescent labelling agents for immune serum. *Amer. J. Path.* **41**, 425.

WICK, G., BEUTNER, E.H. & NILSSON, L.A. (1971) Evaluation of spontaneous autoimmune thyroiditis in chickens of the Obese Strain by defined immunofluorescence. *Ann. N.Y. Acad. Sci.* **177**, 175.

WICK, G. & GRAF, J. (1972) Electron microscopic studies in chickens of the Obese Strain (OS) with spontaneous hereditary autoimmune thyroiditis. *Lab. Invest.* **27**, 400.

WICK, G., KITE, J.H., COLE, R.K. & WITEBSKY, E. (1970a) Spontaneous thyroiditis in the Obese Strain of chickens. III. The effect of bursectomy on the development of the disease. *J. Immunol.* **104**, 45.

WICK, G. & STEINER, R. (1972) Bursectomy and thymectomy of Obese Strain (OS) chickens with spontaneous autoimmune thyroiditis and simultaneous experimental allergic encephalomyelitis. *J. Immunol.* **109**, 1031.

WICK, G., SUNDICK, R.S. & ALBINI, B. (1974) Review: the Obese Strain (OS) of chickens: an animal model with spontaneous autoimmune thyroiditis. *Clin. exp. Immunopathol.* (In press.)

WICK, G., WITEBSKY, E., KITE, J.H. & BEUTNER, E. (1970b) Immunofluorescent studies of thyroid autoantibodies in chickens of the Obese Strain. *Clin. exp. Immunol.* **7**, 173.

WITEBSKY, E., KITE, J. H., WICK, G. & COLE, R.K. (1969) Spontaneous thyroiditis in the Obese Strain of chickens. I. Demonstration of circulating autoantibodies. *J. Immunol.* **103**, 708.