

## Immunofluorescent Findings in Graves' Disease and Hashimoto's Disease

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**Abstract**—Direct immunofluorescent study with antisera against Ig G, A, M, C3 & fibrinogen was performed on thyroid specimens from 21 cases of Graves' disease and 11 cases of Hashimoto's thyroiditis to define immunofluorescent findings in each disorder and their functional significance, with the following results;

1. Ninety five % of Graves' disease cases showed significant granular immunofluorescent deposit along follicular basement membrane and remainder showed scanty granular deposit on interfollicular space.

2. Forty five % of Hashimoto's thyroiditis cases showed immunofluorescent deposit along follicular basement membrane, whereas 63% showed deposition on interfollicular space.

3. Three cases histologically appreciated as Hashimoto's thyroiditis had significant deposit along follicular basement membrane, and all of them showed physical findings and past history compatible with Graves' disease.

4. There was no significant correlation between immunofluorescent deposit, and thyroid function or the presence of thyroid autoantibodies.

5. Comparing immunofluorescent studies with light microscopic findings, degree of immunofluorescent deposit along follicular basement membrane was correlated well with the degree of lymphocytic infiltration in Graves' disease.

In this study, it was shown that the pattern of immunofluorescent deposition in Graves' disease was different from that of Hashimoto's disease, and the degree of immunofluorescent deposit along follicular basement membrane was correlated well with the degree of lymphocytic infiltration in Graves' disease.

**Key Words:** *Immunofluorescent study, Autoimmunity, Graves' disease, Hashimoto's disease*

### INTRODUCTION

Graves' disease and Hashimoto's disease have several features in common. In both disorders, the gland is infiltrated with lymphocytes, frequently organized as germinal center (Warthin *et al.* 1928; Volpe *et al.* 1974), and the two diseases may coexist in the same gland (Friedman and Fialkow 1978a). The cause of Graves' disease and Hashimoto's thyroiditis remains largely unknown but there are many evidences of perturbation in immune system, e.g., presence of thyroid autoanti-

bodies (Mori and Kriss 1971) and sensitization of T-lymphocyte against thyroid antigen (Totterman *et al.* 1979) are observed in both diseases. Moreover, studies on thyroid stimulating immunoglobulin (Onaya *et al.* 1973; Manley *et al.* 1974; Smith and Hall 1974; Mukhtar *et al.* 1975; Smith *et al.* 1977; O'Donnell *et al.* 1973; Totterman *et al.* 1977; Okida *et al.* 1980; Topliss *et al.* 1981) have elucidated the role of autoimmune mechanism in the pathogenesis of these thyroid disorders. Immune complex deposit noticed in immunofluorescent stu-

dies also is listed as one of the evidences to suggest immunologic disturbance as pathogenetic mechanisms (Werner *et al.* 1972; Kalderon *et al.* 1973), but there has been very little information on the correlation of immunofluorescent findings with clinical data.

In this paper, we performed immunofluorescent study of thyroid specimens from 32 patients, and correlated the results with other clinical data to define immunofluorescent findings in each disease and their functional significance.

### MATERIALS AND METHODS

Thyroid specimens were obtained from 32 patients with goiter, comprising 21 cases of pathologically appreciated as diffuse hyperplasia (compatible with Graves' disease) and 11 cases of lymphocytic thyroiditis (compatible with Hashimoto's thyroiditis). Among the 21 cases (16 females and 5 males; mean age 34.7, range 20-61) of pathologically appreciated Graves' disease, 14 patients were euthyroid with antithyroid medication for 2-6 months at the time of biopsy. Eleven cases (all females; mean age 38.3, range 20-58) pathologically appreciated as Hashimoto's thyroiditis were subjected to biopsy without any treatment.

Thirty two thyroid specimens were obtained with needle biopsy and fixed in 10% formalin and embedded in paraffin for histologic study. The specimens were evaluated separately by two pathologists and histologic findings were itemized (Table 1) with grading system (- to +++).

**Table 1.** Histologic criteria used in differentiation of Graves' and Hashimoto's disease

1. Cell destruction	2. Lining cell morphology
3. Oxyphilic cell	4. Lymphoid reaction
5. Fibrosis	6. Follicular size
7. Nuclear polyploidy	8. Scalloping
9. Colloid formation	10. Papillary infolding

Direct immunofluorescent studies were carried out on the 32 biopsied and 3 normal specimens obtained from adjacent tissue of thyroid adenoma on operation as normal controls with antisera against Ig G, A, M, C3 and fibrinogen. Immunofluorescent deposits were categorized into that along follicular basement membrane and that on interfollicular space with grading system (- to +++).

On each patient, thyroid function tests were done at the time of biopsy using radioimmunoassay kits for T3 bead uptake (Abbott, USA), triiodothyronine

(Abbott, USA), thyroxine (Abbott, USA), and thyroid stimulating hormone (Daiichi, Japan). In 16 patients thyroid autoantibodies were checked by hemagglutination technique, namely antimicrosomal antibody (Fuji Zoki, Japan) and antithyroglobulin antibody (Fuji Zoki, Japan). Titers above 1:100 were regarded as positive.

### RESULTS

#### 1. Immunofluorescent findings in Graves' disease and Hashimoto's Thyroiditis

Normal control tissues did not show any significant immunofluorescent deposit in all 3 cases. Twenty out of 21 cases (95%) of Graves' disease showed significant granular immunofluorescent deposits along follicular basement membrane, but only one case (5%) revealed scanty granular deposit on interfollicular space. In Hashimoto's thyroiditis cases, 5 out of 11 patients (45%) showed immunofluorescent deposits along follicular basement membrane, whereas 7 patients (63%) showed deposits on interfollicular space. The major immunofluorescent deposits in both disorders were Ig G and C3 with other components of scanty amount (Table 2). Also Ig G and C3 depositions along follicular basement membrane were correlated positively: specimens with strong Ig G deposition showed significant C3 deposition and *vice versa*.

Three cases histologically appreciated as Hashimoto's disease had significant deposition along follicular basement membrane and all of them showed physical findings and past history pertinent to Graves' disease (Fig. 2). Regarding immunofluorescent deposit on interfollicular space in Hashimoto's disease, the majority were Ig G of focal and smooth pattern without significant C3 deposition.

#### 2. Correlation between immunofluorescent deposit and functional status of thyroid or other clinical data

There were no significant correlation between immunofluorescent deposit and thyroid function studies performed at the time of biopsy (Fig. 3). Also there was no significant difference in the immunofluorescent deposit between untreated hyperthyroid Graves' disease patients and treated euthyroid patients (Fig. 4).

In 16 out of 32 cases, thyroid autoantibodies were checked. Among the 16 checked, antimicrosomal antibody was positive in 7 cases. All the cases with positive antithyroglobulin antibody showed positive antimicrosomal antibody. There was no correlation between the pattern of im-

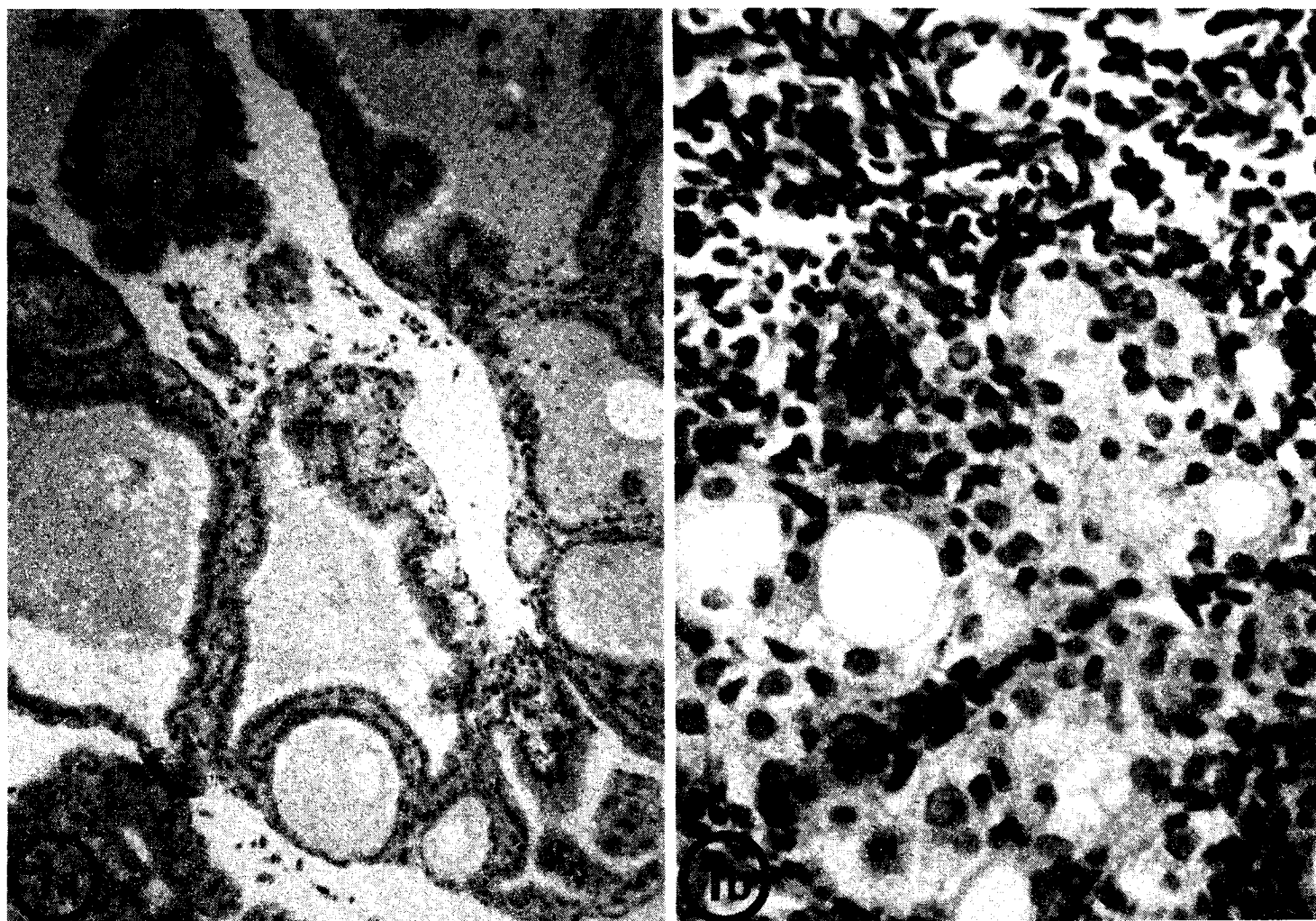


Fig. 1a. Microscopic findings of Graves' disease showing prominent papillary infolding and scalloping suggestive of hyperfunctioning gland, compatible with diffuse hyperplasia (H & E,  $\times 100$ ).

Fig. 1b. Microscopic findings of Hashimoto's disease showing oxyphilic cell in its center with prominent lymphocytic infiltration and fibrosis compatible with lymphocytic thyroiditis (H & E,  $\times 400$ ).

Table 2. The results of immunofluorescent findings in the thyroid gland of Graves' and Hashimoto's disease

Disease	No.	FBM	%	IFS	%	
Graves'	21	20	95	1	5	
Hashimoto's	11	5	45	7	63	
FBM deposit						
	No.	IgG	IgA	IgM	C3	Fbg
Graves'	21	20	2	1	15	6
Hashimoto's	11	5	0	0	3	3
IFS deposit						
	No.	IgG	IgA	IgM	C3	Fbg
Graves'	21	1	0	0	0	1
Hashimoto's	11	6	1	1	1	0

FBM: Follicular basement membrane

IFS: Interfollicular space

Fbg: Fibrinogen

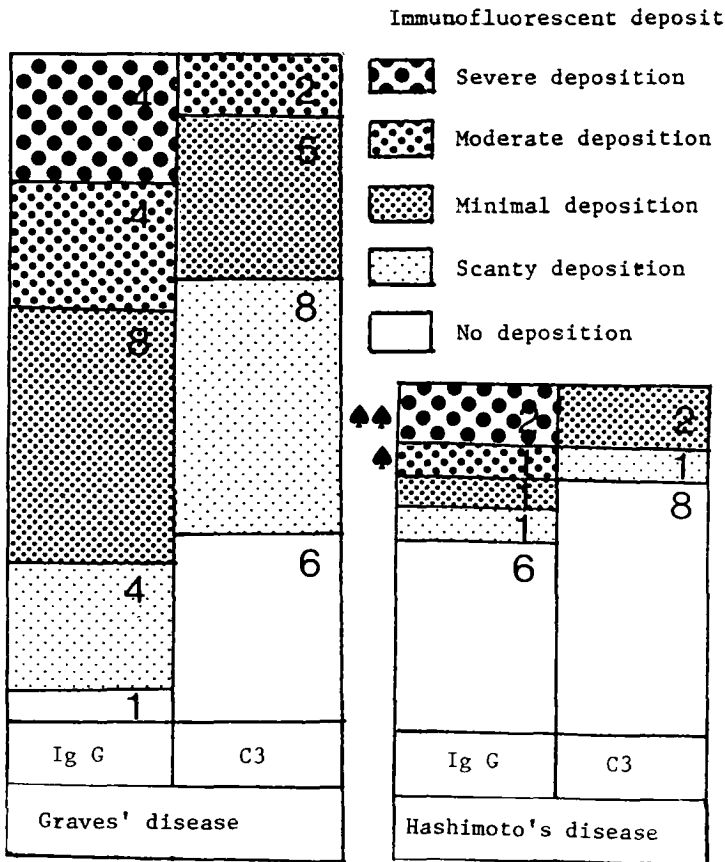


Fig. 2. Deposition of Ig G and C3 along the follicular basement membrane. ♠ Clinically Graves' disease, but histologically appreciated as Hashimoto's disease.

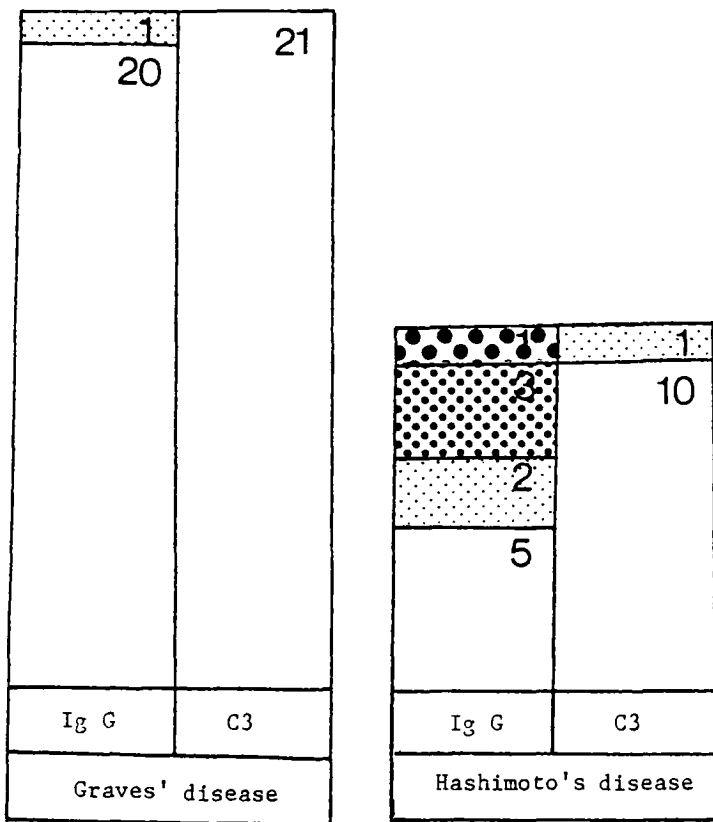


Fig. 3. Deposition of Ig G and C3 on interfollicular space.

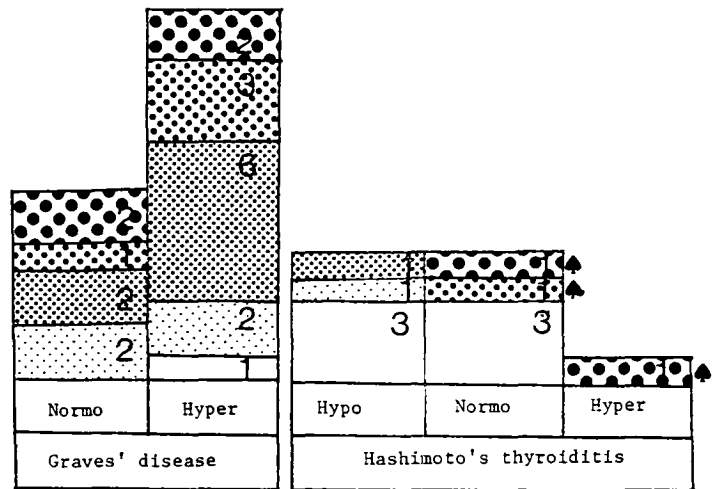


Fig. 4. Correlation between thyroid function and Ig G deposition along the follicular basement membrane

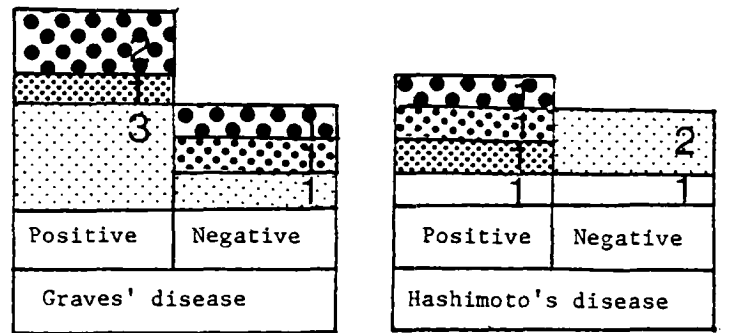


Fig. 5. Correlation between antimicrobial antibody and Ig G deposition along the follicular basement membrane.

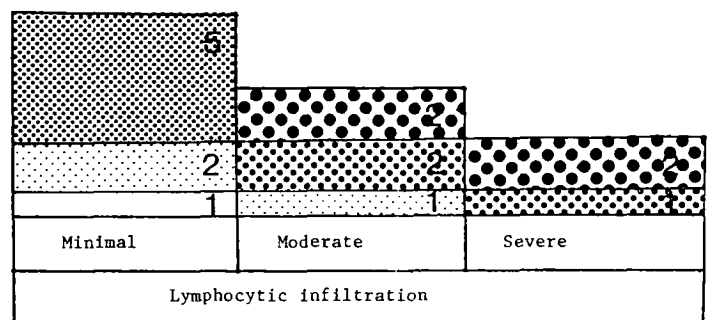


Fig. 6. Correlation between lymphocytic infiltration and immunofluorescent deposit along the follicular basement membrane in Graves' disease.

munofluorescent deposit and the presence of auto-antibodies (Fig. 5).

Comparing immunofluorescent studies with light microscopic studies, the degree of follicular basement membrane deposit was correlated well with the degree of lymphocytic infiltration in Graves' disease (Fig. 6).

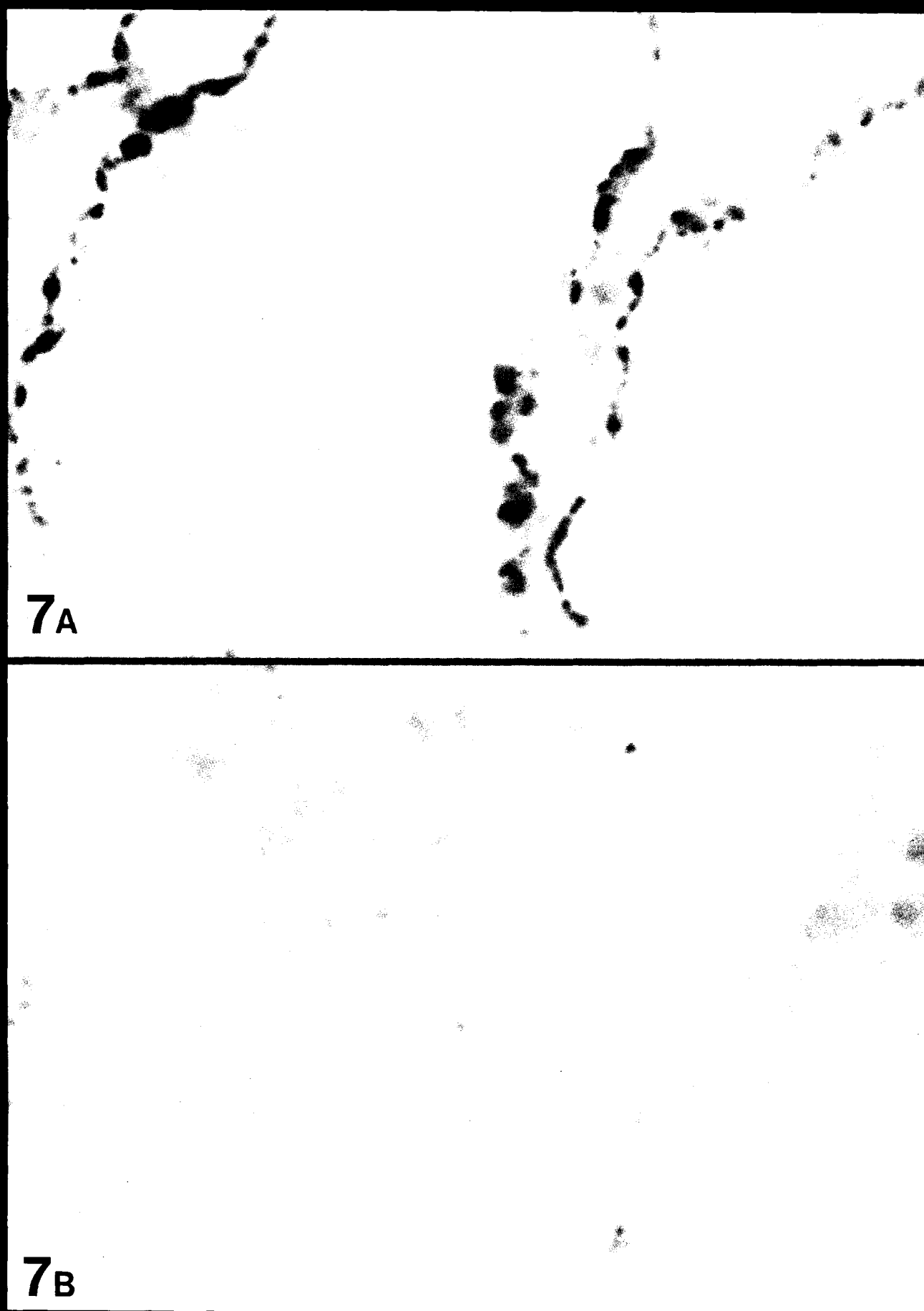


Fig. 7a. Immunofluorescent findings of Graves' disease against Ig G showing coarse granular immune complex along follicular basement membrane ( $\times 100$ ).

Fig. 7b. Immunofluorescent finding of Hashimoto's disease against Ig G showing focal and smooth deposit on interfollicular space with insignificant deposition along follicular basement membrane ( $\times 100$ ).

## DISCUSSION

In Graves' disease, the major immunofluorescent deposit was confined to follicular basement membrane without involvement of interfollicular space (Fig. 7). Also all of the 3 cases which demonstrated strong follicular basement membrane deposits in histologically appreciated Hashimoto's disease showed physical findings and past history compatible with Graves' disease. Concerning the nature of immune complex, it is suggested that antibodies against more than one antigen, especially antimicrosomal antibody and antithyroglobulin antibody are involved. Gamma-globulin eluted from thyroid tissue homogenate of Graves' disease has been characterized by reactivity to thyroglobulin and microsomal antigen (Fujiwara *et al.* 1981), and antithyroglobulin antibody has been identified in thyroid gland as a major component of immune complex in fibrous variant of Hashimoto's thyroiditis (Salabe *et al.* 1974).

Immune complexes capable of tissue damage have been demonstrated in Graves' disease (Fragraeus and Jonsson 1970) and antibody coupling to thyroid cell antigens is the initial step in antibody-dependent lymphocyte-mediated cytotoxicity (Calder *et al.* 1973; Calder and Irvine 1975). Therefore, the fact that the degree of lymphocytic infiltration in Graves' disease is correlated well to the degree of immunofluorescent deposition along follicular basement membrane might suggest that each patient is on the different stage of evolution to a more destructive process especially due to antibody mediated cytotoxicity, and finally to the state of euthyroid or hypothyroid even with maximal exogenous stimuli, *e.g.*, thyroid stimulating immunoglobulin is provided. Also the antimicrosomal antibody is known to be largely directed against thyroid cellular antigens which may play a role in tissue destruction.

There are many aspects in common between Graves' disease and Hashimoto's thyroiditis. Both conditions may aggregate in the same family, and show many common immunologic stigmata such as presence of autoantibodies to thyroid tissue (Mori and Kriss 1971), and lymphocytic infiltrations within thyroid gland itself (Warthin *et al.* 1928; Volpe *et al.* 1974). Despite these close relationships, there are several elements that separate Graves' disease from Hashimoto's thyroiditis. Thyroid stimulating immunoglobulin is very commonly found in most of Graves' disease patients, but only

occasionally in Hashimoto's disease patients (Mukhtar *et al.* 1975; Sugenuya *et al.* 1979). The histocompatibility antigens also appear to differ between these two conditions (Friedman and Fialkow 1978 b, Strakosch *et al.* 1982).

Our observation that Hashimoto's disease has different pattern of immunofluorescent deposit is noteworthy (Fig. 7a & b). Immunofluorescent deposit against Ig G of focal and smooth pattern on interfollicular space in Hashimoto's disease might be rather on the surface on monocytes that bear immunoglobulin attached to their Fc receptor — "armed lymphocytes" with antithyroid antibodies. It could also be the soluble immune complex formed in the process of differentiation of memory cells which reflects the participation of humoral immune system in Hashimoto's disease and is compatible with the report of Wall *et al.* (1983) who demonstrated increased percentage of OKT 1a+ cells in thyroid specimens of Hashimoto's thyroiditis. After all, the difference in the immunofluorescent findings between Graves' disease and Hashimoto's thyroiditis might be the manifestation of different immunologic effector arms on the common spectrum of autoimmune thyroiditis, but needs to be defined in the future.

Our data showed no significant correlation between immunofluorescent deposit and thyroid function status or thyroid autoantibodies. Since the immune complex deposit with complement is likely to cause cytotoxicity (Roitt and Doniach 1967; Calder *et al.* 1973; Calder and Irvine 1975) and immunofluorescent deposition along follicular basement membrane is correlated well with the degree of lymphocytic infiltration, it could be deducible that the discrepancies between immunofluorescent deposition and thyroid functional status should exist. Yoshida *et al.* (1978) noticed positive correlation between the presence of antimicrosomal antibody and lymphocytic infiltration in postmortem autopsied cases. The finding that our data revealed no significant correlation between immunofluorescent deposit and thyroid autoantibodies with positive correlation between immunofluorescent deposition and lymphocytic infiltration could be due to insufficient number of cases checked in the present study or to the difference in the population under study since the population with overt thyroid disease had been excluded in Yoshida's study, but that should be clarified through further observations.

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= 국문초록 =

## Graves 및 Hashimoto병에서의 면역형광소견

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Graves병과 Hashimoto병의 원인은 현재까지도 규명되어 있지는 않지만 각각의 질환에서 면역기전의 이상이 있다는 증거는 상당히 많이 알려져 있다. 특히 최근에 thyroid stimulating immunoglobulin에 관한 연구와 organ specific suppressor T cell defect를 규명하는 일련의 연구들은 자가면역성 갑상선 질환의 병인론에 관한 지식을 훨씬 넓혀 주었다. 그러나 많은 이론, 임상관찰 및 병리 소견 간에는 많은 상이점이 발견되었고 면역형광법에 의한 면역복합체의 침착소견 역시 다른 임상소견들과 서로 비교 연구가 되어 있지 않았다.

저자들은 이에 Graves 환자 21명, Hashimoto병 환자 11명에서 면역형광법에 의한 면역복합체 침착소견을 관찰, 이를 다른 임상소견과 비교하여 다음과 같은 결론을 얻었다.

1. Graves병 환자중 95%에서 여포기저막에 과립성의 면역복합체의 침착이 관찰되었으나 여포간 공간에는 단지 5%만의 과립성 침착이 관찰되었다.
2. 반면에 Hashimoto병 환자에서는 미량의 침착으로 45%에서 여포기저막에 그리고 63%에서 여포간 공간에 촛점상 침착이 관찰되었다.
3. 여포기적막 침착을 보인 Hashimoto병 환자의 60%에서 이학적 소견 및 과거력상 Graves 환자 혹은 환자였음을 알 수 있는 소견을 나타내고 있었다.
4. 면역형광체 침착과 갑상선 기능 그리고 갑상선 자가항체 소견 사이에서는 별다른 상관관계를 발견할 수 없었다.
5. Graves병에서 면역형광체의 여포기저막 침착정도와 임파구 침윤의 정도 사이에는 좋은 상관관계를 보이고 있었다.

결과를 요약하면, Graves병에서는 면역형광체가 여포기저막에 주로 침착하고 있는 다시 임파구 침윤과 좋은 상관관계를 보이고 있으며 Hashimoto병에서는 Graves병과는 달리 여포간 공간에 면역형광체의 침착을 많이 보이고 있었다.